METHOD DEVOLOPMENT AND VALIDATION OF INDAPAMIDE AND PERINDOPRIL ERBUMINE IN BULK AND TABLET DOSAGE FORM

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted

BY

R.GOWTHAM

Reg.No.261330953

Under the guidance of

Prof.Dr.D.Babu Ananth, M.Pharm,Ph.D.,

DEPARTMENT OF PHARMACEUTICAL ANALYSIS,

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

OCT 2015

METHOD DEVOLOPMENT AND VALIDATION OF INDAPAMIDE AND PERINDOPRIL ERBUMINE IN BULK AND TABLET DOSAGE FORM

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted
By

Reg.No. 261330953

DEPARTMENT OF PHARMACEUTICAL ANALYSIS,

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

OCT 2015

Prof.Dr.D.BabuAnanth,M.Pharm., Ph.D.,

Principal,

Edayathangudy .G.S.Pillay College of Pharmacy,

Nagapattinam – 611 002.

CERTIFICATE

This is to certify that the dissertation entitled **"METHOD DEVOLOPMENT AND VALIDATION OF INDAPAMIDE AND PERINDOPRIL ERBUMINE IN BULK AND TABLET DOSAGE FORM"** submitted by **R.GOWTHAM** (Reg No: 261330953) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in Department of Pharmaceutical Analysis, Edayathangudy.G.S.Pillay College of Pharmacy, Nagapattinam**.** during the academic year 2014-2015.

Prof.Dr.D.BabuAnanth,M.Pharm., Ph.D.,

Place: Nagapattinam

Date:

ACKNOWLEDGEMENT

I would like to express profound gratitude to **Jothimani Chevalier Thiru.G.S.Pillay,** Chairman, E.G.S.Pillay College of Pharmacy, and **Thiru.S.Paramesvaran, M.Com., FCCA.,**Secretary, E.G.S.Pillay College of Pharmacy.

I express my sincere and deep sense of gratitude to my guide **Prof.Dr.D.BabuAnanth, M.Pharm, Ph.D.,** Principal, Department of Pharmaceutical Analysis, Edayathangudy.G.S.Pillay College of Pharmacy, for his invaluable and extreme support, encouragement, and co-operation throughout the course of my work.

I express my sincere gratitude to **Prof. Dr.M.Murugan, M.Pharm., Ph.D.,**Director cum Professor, Head, Department of Pharmaceutics. E.G.S.Pillay College of Pharmacy, for his encouragement throughout the course of my work.

I wish to express my great thanks to **Prof.K.Shahul Hameed Maraicar , M.Pharm., (Ph.D),** Director cum Professor , Department of Pharmaceutics, E.G.S.Pillay College of Pharmacy, for his support and valuable guidance during my project work.

I would like to extend my thanks to all the **Teaching Staff** and **Non Teaching Staff**, who are all supported me for the successful completion of my project work.

Last but not least, I express my deep sense of gratitude to my parents, family members and friends for their constant valuable blessings and kindness.

INDEX

\

1. INTRODUCTION

Analytical chemistry is basically concerned with the determination of the chemical composition of matter however, identification of substances, the elucidation of its structure and quantitative analysis of composition, are the aspects covered by modern analytical techniques. By means of analytical techniques both qualitative analysis (the presence or absence of one or more elements) and quantitative analysis (how much amount is present) can be done.

1.1. CHROMATOGRAPHY

The word chromatography is derived from the Greek letters chromos meaning color and the graph means color writing. The initial use of the terms is attributed to T Swett. It can be defined as “a separation process that is achieved by distribution of substance between two phases that is stationary phase and mobile phase.”

IMPORTANCE OF CHROMATOGRAPHY

Chromatography is one of the most powerful and versatile analytical techniques available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in a mixture in a single one analytical run. Its versatility comes from its capacity to handle wide variety of samples that may be gaseous, liquid or solid in nature. The sample can range in complexity from a single substance to a multi component mixture containing widely different chemical species. Another aspect of versatility is that the analysis can be carried out on a very costly complex instrument and on the other hand on a simple inexpensive thin layer plate.

1.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

HPLC is introduced commercially in 1969 and since then it has undergone extensive modifications and innovation which lead to the emergence as the foremost analytical tool for quantitative analysis. HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain a stationary flow rate, liquid must be pressurized to a few thousands of pounds per square inch.

A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONSISTS OF THE FOLLOWING COMPONENTS

- Solvent delivery system
- Pump
- Solvent degassing system
- Gradient elution devices
- Sample introduction system
- Injectors
- Detectors

1.3. METHOD DEVELOPMENT

INTRODUCTION

Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why. In the development stage decisions regarding choice of column, mobile phase, detectors and method of quantitation must be addressed. There are several reasons for developing new methods of analyte:

- There may not be a suitable method for a particular analyte in the specific sample matrix.
- Existing method may be too expensive, time consuming, or energy intensive, or they may not be easily automated.
- Existed method may not provide an adequate sensitivity or analyte selectivity in sample of interest.
- There may be a need for an alternative method to confirm, for legal, or scientific reasons, analytical data originally obtained by existing methods.
- Next, it is necessary to translate the goals of the method into a method development design. Goals for a new or a new improved analytical method might include the following:
- Qualitative identification of the specific analyte (x) of interest providing some structural information to confirm “general behavior.” (eg. Retention time, color change, pH etc)

- Quantitative determination at trace levels when necessary that is accurate, precise and reproducible in any laboratory setting when performed according to established procedures.
- Decreased cost per analysis by using simple quality assurance and quality control procedures.
- Sample preparations that minimize time, effort, materials and volume of sample consumed.

1.3.1. METHOD DEVELOPMENT IN HPLC INVOLVES THE FOLLOWING STEPS:

Step 1: Method selection

The method selection should contain

- Solubility profile - which includes solubility of drug substance in different solvents and at different pH conditions
- Analytical profile - which include analytical profile of the drug substance, impurity and degradation products
- Solubility profile - which includes stability profile of the drug substance with respect to the storage conditions

Step 2: Selection of initial conditions

This step determines the optimum conditions to adequately retain all analytes i.e., ensure no analytes has capacity factor of less than 0.5 (poor retention could result in peak overlapping) and no analyte has capacity factor 10-15 (excessive retention leads to long analysis time and broad peaks with poor detectability).

Step 3: Selectivity optimization

The aim of this step is to achieve adequate selectivity (peak spacing). The mobile phase and stationary phase compositions need to be taken into account. To minimize the number of trail chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be considered. The optimization of mobile phase parameters is always considered first as that is much easier and convenient than stationary phase optimization.

Step 4: System optimization

This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing, particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

Step 5: Method Validation

Proper validation of analytical methods is important for pharmaceutical analysis when ensuring of the continuing efficacy and safety of each batch manufactured relies solely in the determination of quality. Method validation is generally a one-time process performed after the method has been developed to demonstrate that the method is scientifically sound and that it serves the intended analytical purpose.

1.3.2. METHOD OPTIMIZATION

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts, asymmetry, capacity, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.

Optimization of a method can follow either of two general approaches:

- Manual
- Computer driven

The various parameters that include to be optimized during method development

- Mode of separation
- Selection of stationary phase
- Selection of mobile phase
- Selection of detector.

1.3.2.1. Selection of mode of separation

In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A second factor is the nature of the matrix.

1.3.2.2. Selection of stationary phase/ column

Selection of the column is the first and the most important step in method development because the column is the heart of the separation process.

- Length and diameter of the column
- Nature of the packing material
- Shape of the particles
- Size of the particles
- Percentage of Carbon loading
- Pore volume
- Surface volume
- End capping

1.3.2.3. Selection of separation system/ column

The column is selected depending on the nature of the solute. Reversed phase mode of chromatography facilitates a wide range of columns like dimethyl silane (C_2), butylsilane (C_4), octylsilane (C_8), octadecylsilane (C_{18}), base deactivated silane (C_{18}) BDS phenyl, cyanopropyl (CN), nitro, amino etc. C_{18} was chosen for this study since it is most retentive one.

Peak shape is equally important in method development. Columns that provide symmetrical peaks are always preferred. While peaks with poor asymmetry can result in:

- Inaccurate plate number and resolution measurement
- Imprecise quantitation
- Degraded and undetected minor bands in the peak tail
- Poor retention reproducibility

A column which gives separation of all the impurities and degradants from each other and from analyte peak and which is rugged for variation in mobile phase shall be selected.

1.3.2.4. Selection of mobile phase

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from each other and from analyte peak.

In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute – stationary phase. For a given stationary phase, the retention of the given solute depends directly upon the mobile phase, the composition of which has to be selected in order to get appropriate and required solute retention. The mobile phase has to be adopted in terms of elution strength (solute retention) and solvent selectivity (solute separation).

The following are the parameters, which shall be taken into consideration while selecting and optimizing the mobile phase:

- Buffer
- pH of the buffer
- Mobile phase composition

1.3.2.5. Selection of detector

The detector was chosen depending upon some characteristics property of the analyte like UV absorbance, fluorescence, conductance, oxidation, reduction etc.

Characteristics that are to be fulfilled by a detector to be used in HPLC determination are:

- High sensitivity
- Facilitating trace analysis
- Negligible base line noise
- To facilitate lower detection
- Larger linear dynamic range
- Lower dead volume
- Non destructive to sample
- Inexpensive to purchase and operate

1.4 ANALYTICAL PARAMETERS AS PER ICH GUIDELINES

The objective of validation of analytical procedure is to demonstrate that it is suitable for its intended purpose.

1.4.1. TYPES OF ANALYTICAL PARAMETERS

The objective of validation of analytical procedures is directed to the most common types of analytical procedures:

- Identification tests
- Quantitative tests for impurities content
- Limit tests for control of impurities

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Range
- Specificity
- Linearity
- Detection Limit
- Quantification Limit
- Ruggedness
- Robustness

1.4.1.1.Accuracy

The closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

1.4.1.2. Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions. A more comprehensive definition proposed by the ICH divides precision into three types.

- Repeatability

- Intermediate Precision
- Reproducibility

- Repeatability

Repeatability is the precision of the method under the complete operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed as intra-assay precision and involves multiple measurements of the same sample by the same analyte under the same conditions.

- Intermediate Precision

Intermediate precision is the agreement of complete measurements when the same method is applied many times within the same laboratory. This can include full analysis on different days, instruments or analytes, but would involve multiple preparations of samples and standards.

- Reproducibility

Reproducibility examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

Precision often is expressed by the standard deviation or relative standard deviation of the dataset.

1.4.1.3. Range

The range of a method can be defined as the upper and lower concentration for which the analytical method has adequate accuracy, precision and linearity. The range of concentration examined will depend on the type of method and its use.

1.4.1.4. Specificity

Specificity is the ability to assess the analyte in the presence of components, which may be expected to be present. Assuring specificity is the first step in developing and validating a good method.

1.4.1.5. Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

1.4.1.6.Detection Limit:

The Detection Limit of an individual analyte procedure is the lower amount of analyte in a sample which can be detected but not necessarily qualitated as an exact value. The detection limit (LOD) may be expressed as:

$$LOD = 3.3\sigma/S$$

where , σ = the standard deviation of the response

S = the slope of the calibration curve (of the analyte)

1.4.1.7. Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

1.4.1.8. Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters.” The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters.

1.5 System suitability parameters:

System suitability of the methods is performed by calculating the chromatographic parameters namely column efficiency, resolution, peak asymmetry factor and capacity factor on the repetitive injection of standard substances using the following formula:

Retention time (R_t) is the time of elution of peak maximum after injection of sample.

Resolution (R_s) is the difference between the retention times of two solutes divided by their average peak width. The ideal value of (R_s) is 1.5

$$R_s = (tR_2 - tR_1)/(W_1 + W_2)/2$$

Where R_{t1} and R_{t2} are the retention times of component 1 and 2,

W_1 and W_2 are peak widths of components 1 and 2 respectively.

Column Efficiency (N) or Band broadening of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Column with N ranging from 5000 to 100000 plates/ meter are ideal for a good separation.

$$N = 16(t/w)^2 \quad \text{or}$$

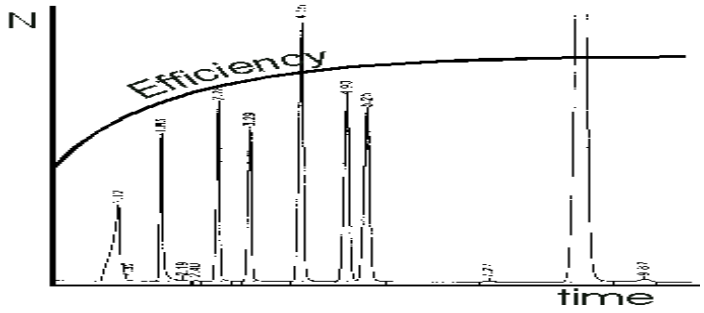
$$N = 5.54(t/w^{1/2})^2$$

Where,

N = width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the Baseline.

W1/2 = width of the peak at half height, obtained directly by electronic integrators.

$$N = \left(\frac{t_R}{\sigma_{total}} \right)^2 \qquad \sigma_{total} = \sigma_{column} + \sigma_{inject.} + \sigma_{cell} + \sigma_{connect.}$$



Capacity factor (k') is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of (k') ranges from 2-10.

$$k' = V_1 - V_0 / V_0$$

Where, V_1 is the retention volume at the apex of the peak (solute)

V_2 is the void volume of the system.

Selectivity (∞) is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency. The ideal value of ∞ is 2.

$$\text{Selectivity } (\infty) = V_2 - V_0 / V_1 - V_0 = k'_1 / k'_2$$

Where V_0 is the void volume of the column

V_2 and V_1 are the retention volumes of the second and first peaks, respectively.

Peak Asymmetry Factor (A_s) can be used as a criteria of column performance. For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

$$A_s = b/a$$

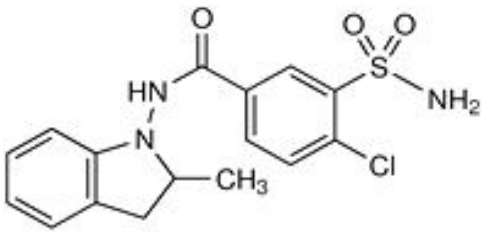
Where a & b are the distances on either side of the peak midpoint.

2. DRUG PROFILE

2.1 INDAPAMIDE

DRUG NAME : Indapamide

CHEMICAL STRUCTURE :



CHEMICAL FORMULA : $C_{16}H_{16}ClN_3O_3S$

MOLECULAR WEIGHT : 365.8 g/mol

IUPAC NAME : 4-chloro-N-[(2RS)-2-methyl-2,3-dihydro-1H-indol-1-yl-3-sulphamoyl benzamide.

DESCRIPTION : White or Almost white powder.

SOLUBILITY : Partially insoluble in water, soluble in ethanol (96 per cent)

CATEGORY : Thiazide like Diuretic.

MELTING POINT : 185°C

DOSAGE AND ADMINISTRATION: 1.25 mg once a day to control high pressure.

MECHANISM OF ACTION: Indapamide is a new oral antihypertensive diuretic and antihypertensive agent. The drug appears to have a unique mechanism of action, combining diuretic effects with a direct vascular action, presumably secondary to calcium channel blockade. Accordingly, calcium

channel blockers, block influx of extracellular calcium through ion selective channels in the membrane both in cardiac and smooth muscle.

The administration of a fixed combination of perindopril and indapamide to a broad range of patients with type 2 diabetes mellitus is associated with reduced risks of major vascular events, including death.

ADVERSE EFFECTS:

- dry mouth, thirst, nausea, vomiting;
- feeling weak, drowsy, restless;
- fast or uneven heartbeat;
- Muscle pain or weakness.

BRAND NAME :

Lozol

Apo-indapamide

Natrix

Natilix

OFFICIAL :

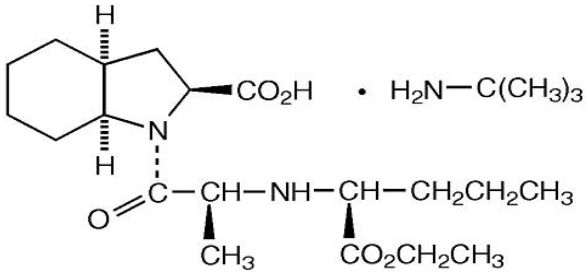
British pharmacopoeia.

United State Pharmacopoeia.

2.2 PERINDOPRIL ERBUMINE

DRUG NAME : Perindopril erbumine

CHEMICAL STRUCTURE :



CHEMICAL FORMULA : $C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$

MOLECULAR WEIGHT : 441.6 g/mol

IUPAC NAME : 2-methylpropan-2-amine (2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1- (ethoxy carbonyl) butyl] amino]propanoyl]octahydro-1H-Indol-2-carboxylate.

DESCRIPTION : White or Almost white, slightly hygroscopic, crystalline powder.

SOLUBILITY : Freely soluble in water and in ethanol (96 per cent), soluble or sparingly soluble in methylene chloride.

CATEGORY : Angiotensin converting Enzyme Inhibitor

MELTING POINT : 537.4°C

DOSAGE AND ADMINISTRATION: Perindopril is taken in 4 mg or 8 mg

MECHANISM OF ACTION : Perindopril is the free acid form of perindopril erbumine, is a pro-drug and metabolized in vivo by hydrolysis of the ester group to form perindoprilat, the biologically active metabolite, inhibits ACE in human subjects and animals. The Perindoprilat lowers blood pressure by inhibition of ACE activity. ACE is a peptidyl dipeptidase that catalyzes conversion of the inactive decapeptide, angiotensin I, to the vasoconstrictor, angiotensin II. Angiotensin II is a potent peripheral vasoconstrictor, which stimulates aldosterone secretion by the adrenal cortex, and provides negative feedback on renin secretion. Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction, increased plasma renin activity and decreased aldosterone secretion. The latter results in diuresis and natriuresis and may be associated with a small increase of serum potassium.

ADVERSE EFFECTS:

- Cough
- fatigue
- headache
- disturbances of mood and/or sleep

BRAND NAME:

Aceon

Apo-perindopril

Coversyl

OFFICIAL:

British Pharmacopoeia.

3. LITERATURE REVIEW

3.1. Jignesh *et al.,*(2011), performed Absorption factor method for perindopril erbumine and amlodipine besylate at wavelength maxima 215 nm and 237 nm respectively. Amlodipine besylate was shown linear at 237 nm but Amlodipine besylate also showed absorbance at 215nm and given interference in determination of Perindopril erbumine. Quantitative estimation of Perindopril erbumine was carried out by subtracting interference of Amlodipine besylate using experimentally calculated absorption factor.

3.2. Darshana kartilal modi *et al.,*(2011), developed simultaneous equation method for the measurement of absorbances at two wavelengths 210.4nm (λmax of perindopril) and 241.2nm (λmax of indapamide) in methanol. The linearity was observed in the concentration range of 24 – 56 µg mL^{-1} for perindopril and 7.5 – 17.5 µg mL^{-1} for indapamide. The method showed good reproducibility and recovery with % RSD less than 2.

3.3. Juddy joseph *et al.,* (2011), proposed the Simultaneous Estimation of Perindopril erbumine and Indapamide by RP-HPLC in Pharmaceutical Dosage forms. The determination was carried for a runtime of 20min at 40˚C on Inertsil ODS-3V column having 250mm x 4.6mm with 5µm particle size and potassium dihydrogen phosphate buffer adjusted to pH 3.0 using ortho phosphoric acid and acetonitrile (60:40 v/v) was used as eluent at a constant flow rate of 1.0ml/min with UV detection wavelength of 215nm. The retention time of PE and ID was about 11.9 and 4.9min with correlation coefficient of 0.9992 and 0.9990 respectively. The linearity was established at 8-24µg/ml for PE and 2.5-7.5µg/ml for ID and the mean recovery for both drugs were found to be 100.3% at a load volume of 50µl.

3.4. A.K. Pathak *et al.,* (2011), developed Chromatographic separation on Perindopril and Indapamide by using BDS hypersil C18 column (25cm×4.6mm, 5 µm) with a mobile phase comprising of mixture of potassium dihydrogen phosphate buffer (pH 2.6) and acetonitrile (65:35) at a

flow rate of 1.5 ml/min with detection at 210 nm. Separation was completed in less than 15 min.

3.5.Patel Amit R. *et al.,* (2011), performed simultaneous estimation. The separation was achieved on Telmisartan and Indapamide by RP-HPLC by using an Amazone C18, 5 µm, 150 x 4.6 mm the mobile phase (Buffer: acetonitrile: methanol) (45+25+30) Potassium dihydrogen phosphate & Triethaylamine pH 3.0 with ortho phosphoric acid buffer flow rate of 1 ml/min and UV detection at 285 nm. Comprehensive stress testing of telmisartan and indapamide Rt= 4.7 min, 10.7 min was according to the ICH guideline Q1A (R2). The linearity of the proposed method was in the range of 6-22.5 microg/mL (r2 = 0.999) for telmisartan and 11.2-42 µg/ml (r2 = 0.9997) for indapamide.

3.6. C.Koza, *et al.,* (2011), has developed HPLC method involved by using HiQSil-C-18W ODS, (250 mm × 4.5 mm 5µm), column and mobile phase was ACN: water in proportion of 50:50 v/v, pH adjusted to 3.2 ± 0.1 with 1 % o-phosphoric acid. The flow rate was 1.0 mLmin-1 and effluent was monitored at 210 nm. The retention time of Losartan potassium and perindopril erbumine were eluted at 6.7 min and 4.5 min respectively. The method was found to be linear in the range of 2-18 g/l for both the drug. The coefficient of variance for both the drug was more than 0.999. The mean percentage recovery was found to be 98.40 % for Losartan potassium and 97.50 % for perindopril erbumine. The limits of quantification of Losartan potassium and perindopril erbumine were found to be 0.109/gmL-1 and 0.041/gmL-1.

3.7. Mohit G Dewani *et al.,* (2011), proposed the simultaneous estimation of Indapamide and Perindopril by HPTLC, here the plates precoated with silica gel 60 F254 were used. The mobile phase used was Dichloromethane: Methanol : Glacial acetic acid in the ratio of 9.5:0.5:0.1 v/v/v. Both the drugs showed considerable absorbance at 215 nm. Linearity was obtained in the concentration range of 1-5 µg/band and 100-500 ng/band for perindopril and indapamide respectively.

3.8. Jodia *et al.,* (2010), performed the stability indicating assay method for simultaneous determination of Perindopril and Indapamide by RP-HPLC and the separation was achieved on an XTerra RP18, 5 microm, 150 x 4.6 mm id column at 55 °C by using the mobile phase sodium dihydrogen phosphate buffer (pH 2.0; 0.005 M) acetonitrile (75 + 25, v/v) at a flow rate of 1 ml/min and UV detection at 215 nm. Comprehensive stress testing of perindopril and indapamide was carried out according to the ICH guideline Q1A (R^2). The specificity of the method was determined by assessing interference from the placebo and by stress testing of the drug (forced degradation).The linearity of the proposed method was investigated in the range of 24-56 microg/mL (r2 = 0.9993) for perindopril and 7.5-17.5 µg/m(r2=0.9992) for indapamide.

3.9. Abdalla Elshawana *et al.,* (2009), developed the LC method for the determination of Perindopril and Indapamide. The drugs were separated at room temperature on a 250 mm × 4.6 mm, 5-µm particle size, cyanopropyl column with 10 mM KH_2PO_4, pH 6.0-methanol 55:45 (*v*/*v*) as mobile phase at a flow rate of 1 ml min^{-1}. Detection was at 210 nm.

4. AIM AND OBJECTIVE

4.1. AIM:

The aim of the work is to develop a precise, accurate, simple and reliable, less time consuming validated RP-PLC method for Indapamide and Perindopril erbumine in bulk and tablet dosage form.

4.2. OBJECTIVE:

- To develop new, simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Indapamide and Perindopril erbumine.
- To validate the proposed method in accordance with ICH guidelines for the intended analytical application.
- To apply the proposed method for analysis of these drugs in their combined dosage form.

4. AIM AND OBJECTIVE

4.1. AIM:

The aim of the work is to develop a precise, accurate, simple and reliable, less time consuming validated RP-PLC method for Indapamide and Perindopril erbumine in bulk and tablet dosage form.

4.2. OBJECTIVE:

- To develop new, simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Indapamide and Perindopril erbumine.
- To validate the proposed method in accordance with ICH guidelines for the intended analytical application.
- To apply the proposed method for analysis of these drugs in their combined dosage form.

6. EXPERIMENTAL WORK

6.1. ANALYTICAL METHOD DEVELOPMENT FOR THE ESTIMATION OF INDAPAMIDE AND PERINDOPRIL ERBUMINE BY RP-HPLC METHOD:

Instruments used

HPLC Instrument	Shimadzu LC 10 AT pump Spinchrome software UV detector SPD 10A
Injector	Rheodyne
Column	YMC column, 150 x 4.6mm, 3µ
UV Spectrophotometer	Thermo electron corporation
pH meter	Adwa-AD 1020
Electronic Balance	AFROSET FX-400
Ultra sonicator	Elma S 300H
Pipettes, burettes, beakers	Borosil

Chemicals and Reagents used

1. Indapamide
2. Perindopril erbumine
3. Water HPLC grade
4. Acetonitrile HPLC grade
5. Ammonium dihydrogen phosphate
6. Ortho phosphoric acid

6.1.1. Selection of Mobile Phase:

Indapamide and Perindopril erbumine are marketed as combined dosage formulation. The proposed method for the estimation of Indapamide and Perindopril erbumine required adequate resolution of the two drug peaks in the chromatogram. Several solvent systems were tried to obtain good optimum resolution.

a) Separation using potassium dihydrogen phosphate : dipotassium hydrogen phosphate : acetonitrile (pH 3 using ortho phosphoric acid) (40:60v/v)

In this composition when drug was injected with isocratic programming, it was observed that perindopril erbumine peak was eluted in the void volume peaks.

b) Separation using sodium dihydrogen phosphate : disodium hydrogen phosphate : acetonitrile (pH 3 using ortho phosphoric acid) (40:60v/v)

In this composition when injected, perindopril erbumine was still getting eluted in the void volume peak and the resolution between the peaks was too less.

C) Separation using sodium dihydrogen phosphate : acetonitrile (pH 3 using ortho phosphoric acid) (40:60v/v)

In this combination also, perindopril erbumine peak was eluted in the void volume peak and the resolution between the peaks was too less.

D) Separation using Ammonium dihydrogen phosphate : acetonitrile (pH 2.5 using ortho phosphoric acid) (60:40v/v)

By using this combination Indapamide and Perindopril erbumine peaks were separated properly with good resolution meeting all the requirements.

6.1.2. Selection of wavelength for detection

Standard stock solution of Indapamide

Accurately weighed 100mg of Indapamide and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of diluents. The volume made up to 100ml with diluents to obtain the concentration of 1000µg/ml.

Working standard solution

0.1ml of stock solution was further diluted in a 10ml volumetric flask with mobile phase to get a concentration of 10µg/ml.

Standard stock solution of Perindopril erbumine

Accurately weighed 100mg of Perindopril erbumine and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of diluents. The volume made up to 100ml with diluents to obtain the concentration of 1000µg/ml.

Working standard solution

0.1ml of stock solution was further diluted in a 10ml volumetric flask with mobile phase to get a concentration of 10µg/ml.

Preparation of mobile phase

A mixture of 60 volumes of buffer solution (prepared by dissolving 1.1503gm of Ammonium dihydrogen phosphate in 1000 ml of water and adjust the pH to 2.5 with orthophosphoric acid) and 40 volumes of Acetonitrile. The solution was filtered using 0.45 µm membrane filter paper and degassed.

Procedure

Indapamide 10µg/ml solution and Perindopril erbumine 10µg/ml solution were scanned individually on a UV-visible spectrophotometer in the wavelength range of 200 to 400 nm.

The sensitivity of method that uses UV detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both drugs to be detected.

Hence a common maximum of 230 nm at which the mixture of drugs showing maximum intensity was selected.

6.1.3. Optimization of column

The method was performed with various columns like C_{18} column, hypersil column, lichrosorb and inertsil ODS column. YMC (150 x 4.6mm, 3µ) was found to be very ideal as it gave a good peak shape and resolution.

6.1.4. Effect of column temperature

To improve the peak resolution different temperature conditions were tried i.e., 30,35 & 40^0c. The best resolution was found at column temperature 40^0c. so, the optimum temperature was kept constant at 40 ºC i.e., ambient temperature.

6.1.5. Optimized Chromatographic conditions

Instrument : HPLC Shimadzu Separation Module LC-20AT

Prominence liquid chromatograph

Mobile Phase : Ammonium dihydrogen phosphate : Acetonitrile
Mobile Phase Ratio : 60 : 40 v/v
Diluents : Mobile phase
Column : YMC Column (150 x 4.6mm, 3µ particle size)
Detector : 230 nm
Flow rate : 1 ml/min
Runtime : 10 min
Injection volume : 20 µl
Column oven Temperature : 40^oc

Inference:

By following the above conditions, both the peaks were eluting with required resolution and baseline with good signal to noise ratio. All the peaks are well distributed throughout the run time. Blank interference and peak asymmetry were minimized.

6.1.6. Determination of Retention time

Standard stock solution of Indapamide

Accurately weighed 100mg of Indapamide and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of diluents. The volume made up to 100ml with diluents to obtain the concentration of 1000µg/ml.

Working standard solution

10 ml of stock solution was further diluted in a 50 ml volumetric flask with mobile phase to get a concentration of 200µg/ml.

Standard stock solution of Perindopril erbumine

Accurately weighed 100mg of Perindopril erbumine and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of diluents. The volume made up to 100ml with diluents to obtain the concentration of 1000µg/ml.

Working standard solution

10 ml of stock solution was further diluted in a 50 ml volumetric flask with mobile phase to get a concentration of 200µg/ml.

Preparation of mobile phase

A mixture of 60 volumes of buffer solution (prepared by dissolving 1.1503gm of Ammonium dihydrogen phosphate in 1000 ml of water and adjusted the pH to 2.5 with ortho phosphoric acid) and 40 volumes of Acetonitrile. Filter the solution with 0.45 µm filter paper and degassed.

Procedure

20µl of each of these working standard solutions of Indapamide and Perindopril erbumine were injected separately with flow rate of 1 ml/min. Peak area and retention time in chromatogram was observed, recorded in figure1.

6.2. VALIDATION OF DEVELOPED HPLC METHOD FOR ESTIMATION OF INDAPAMIDE AND PERINDOPRIL ERBUMINE

A HPLC method had been developed for the simultaneous estimation of Indapamide and Perindopril erbumine using YMC column (150 x 4.6mm, 3µm), mobile phase Acetonitrile : Buffer (40 : 60 v/v), pH of buffer adjusted to 2.5 with ortho phosphoric acid, detection wavelength at 230 nm at flow rate 1 ml/min, at retention time 4.32 for indapamide and 2.31 for perindopril erbumine.

6.2.1. System suitability

These tests were based on the concept that the equipment, electronics, analytical operations and samples to be analysed which constitutes an integral system that can be evaluated as such. This test ensures that the analytical system was working properly and can give accurate and precise results.

Preparation of Standard Stock Solution:

Accurately weighed and transferred 100mg of Indapamide and 50mg Perindopril erbumine working standard and transferred into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase.

Preparation of working standard solution

100 ml of standard stock solution was taken in a 50 ml volumetric flask and the volume was made up to 50 ml with mobile phase, to get a concentration of 200µg/ml.

Procedure

Standard solution preparation was injected into chromatographic system and chromatograms were recorded (figure 2) and tabulated (table 1)

Acceptance criteria

1. Tailing factor should be Not More Than 2.0.
2. Theoretical plates should be Not Less Than 2000.

6.2.2. Linearity:

The linearity of the analytical method constitutes its ability to elicit test results which are directly proportional to the concentration of the analyte in the sample.

Preparation of Standard Stock Solution:

Accurately weighed and transferred 100mg of Indapamide and 50mg Perindopril erbumine working standard and transferred into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase.

Preparation of Level – I (20mcg solution of Indapamide and Perindopril erbumine):

0.2ml of stock solution was taken in 10ml of volumetric flask, which was diluted up to the mark with mobile phase.

Preparation of Level – II (40mcg solution of Indapamide and Perindopril erbumine):

0.4ml of stock solution was taken in 10ml of volumetric flask, which was diluted up to the mark with mobile phase.

Preparation of Level – III (60mcg solution of Indapamide and Perindopril erbumine):

0.6ml of stock solution was taken in 10ml of volumetric flask, which was diluted up to the mark with mobile phase.

Preparation of Level – IV (80mcg solution of Indapamide and Perindopril erbumine):

0.8ml of stock solution was taken in 10ml of volumetric flask, which was diluted up to the mark with mobile phase.

Preparation of Level – V (100mcg solution of Indapamide and Perindopril erbumine)

1.0ml of stock solution was taken in 10ml of volumetric flask, which was diluted up to the mark with mobile phase.

Preparation of Level – VI (120mcg solution of Indapamide and Perindopril erbumine)

1.2ml of stock solution was taken in 10ml of volumetric flask, which was diluted up to the mark with mobile phase.

Procedure:

Solution of different concentrations (level I - VI) were injected into the chromatographic system and measured the peak area. Chromatogram for Linearity of Indapamide and Perindopril erbumine from Level I — VI are given in Figure 5, 6, 7, 8, 9, 10 and Chromatogram Linearity report was given (figure11).

A calibration curves were plotted for linearity having concentration against peak area and calculated the correlation coefficient (figure 3, 4).

The results were tabulated (table 2, 3, 4)

6.2.3. Assay:

A solution of 20µl standard, sample separately were injected into the chromatographic system and the peak areas of the Indapamide and Perindopril erbumine were measured and the percentage assay was calculated by using the formulae. Chromatograms were recorded (figure 34, 35) and tabulated (table 13, 14), measured the peak responses.

$$\text{Assay percentage} = \frac{A_t}{\text{-------}} \times \frac{W_s}{\text{------}} \times \frac{D_T}{\text{------}} \times \frac{P}{\text{------}} \times \frac{\text{Avg. Wt}}{\text{----------}} \times 100$$

$$\frac{A_s}{} \quad \frac{D_s}{} \quad \frac{W_t}{} \quad 100 \quad \text{Label Claim}$$

Where:

A_t = average area counts of sample preparation.

As = average area counts of standard preparation.

W = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label claim

D_T = Dilution factor of test sample.

6.2.4. Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often expressed as percent recovery by the assay of known added amounts of analyte.

Preparation of Standard stock solution:

Accurately weighed 100mg of Indapamide and 50mg Perindopril erbumine working standard was transferred into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made upto volume with mobile phase.

Procedure:

The standard solutions of concentrations 8.55µg/ml, 10.45µg/ml, 12.35µg/ml of Indapamide and 22.5µg/ml, 27.5µg/ml, 32.5µg/ml of Perindopril erbumine were injected into chromatographic system. Calculated the amount found and amount added for Indapamide and Perindopril erbumine and also calculated the individual recovery and mean recovery values. The Chromatograms were recorded (figure 12, 13, 14, 15). The results were tabulated (table 5, 6)

6.2.5. System Precision:

The system precision was checked by using standard chemical substance to ensure that the analytical system was working properly. The retention time and area of six determinations was measured and percentage Relative Standard deviation was calculated.

Preparation of Standard stock solution:

Accurately weighed 100mg of Indapamide and 50mg perindopril erbumine working standard transferred into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The percentage Relative Standard Deviation for the area of five injections was found to be within the specified limits. The Chromatograms were recorded (figure 16, 17, 18, 19, 20) and the results were tabulated (table 7, 8)

Standard Deviation

$$S.D\ (\sigma) = \sqrt{\frac{\sum (x - x_i)^2}{n-1}}$$

Where, x=Sample,

x_i = Mean value of samples.

n=number of samples.

Coefficient of variance / Relative standard deviation

$$C.V = \frac{\text{Standard Deviation}}{\text{Mean}} \times$$

Acceptance Criteria:

The % RSD for the retention time of five standard injections results should be NMT1%.

The % RSD for the area of five standard injections results should be NMT2%.

6.2.6. Method precision (Repeatability)

Method precision indicates whether a method is giving consistent results for a single batch, usually applied to standardization of methodology.

Preparation of Standard stock solution:

Accurately weighed 100mg of Indapamide and 50mg perindopril erbumine working standard transferred into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made upto volume with mobile phase.

Procedure:

The standard solution was injected for five times and chromatograms were recorded in HPLC. The percentage Relative Standard Deviation was calculated for concentration of drug in triplicates. The results obtained were reported (figure21, 22, 23, 24, 25) and tabulated (table 9, 10)

Acceptance Criteria

The % RSD value for assay should be NMT 2%

6.2.7. Robustness

The Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of the proposed method was determined by analysis of aliquotes from homogenous lots by differing physical parameters like

volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

(a) Effect of variation of Flow rate:

A study was conducted to determine the effect of variation in flow rate by injecting 0.9and 1.1ml/min. Standard solution was prepared and injected into the HPLC system. The retention time values were measured.

(b) Effect of variation of wavelength:

A study was conducted to determine the effect of variation in wavelength. Standard solution was prepared and injected into the HPLC system at 218nm and 224nm. The effect of variation in wavelength was evaluated.

The results were tabulated (table 11) and recoded (figure 26, 27, 28, 29)

Acceptance criteria:

1. Tailing factor should be NMT 2.0 for variation in flow rate.
2. The %RSD should be NMT 2.0 for variation in flow rate.
3. Tailing factor should be NMT2.0 for variation in wavelength.
4. The %RSD should be NMT2.0 for variation in wavelength.

6.2.8. Ruggedness:

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results were reproducible under differences in conditions, analysts and instruments. Hence the proposed method was found to be rugged. The Chromatograms were recorded (figure 30, 31).The results were tabulated (table 12).

Preparation of Stock Solution:

Accurately weighed 100mg of Indapamide and 50mg Perindopril erbumine working standard transferred into a 100ml clean dry volumetric flask

containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase.

From the above solution 1.0ml was taken and made up to 10ml using mobile phase (100mcg).

Procedure:

The standard solution (100mcg) was injected for by different analysts and the area for injections in HPLC was measured. The percentage Relative Standard Deviation for the area of replicate injections was found to be within the specified limits.

6.2.9. Specificity

Specificity is the ability to access unequivocally that the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradation products and matrix components.

Preparation of standard stock solution

Accurately weighed standard drugs Indapamide and Perindopril erbumine 100 mg each was transferred in clean, dry 100 ml volumetric flask and dissolved in few ml of diluents. The volume made up to 100 ml with diluents to obtain the concentration of 1000µg/ml.

Preparation of working standard solution

10 ml of standard stock solution of Indapamide and Perindopril erbumine were taken separately into 50 ml volumetric flask and the volume was made up to 50 ml using mobile phase to get the concentration of 200µg/ml.

Procedure

20µl of each solution of mobile phase, Indapamide and Perindopril erbumine working standard solutions were injected into the chromatographic system and chromatograms obtained are recorded (figure 32, 33)

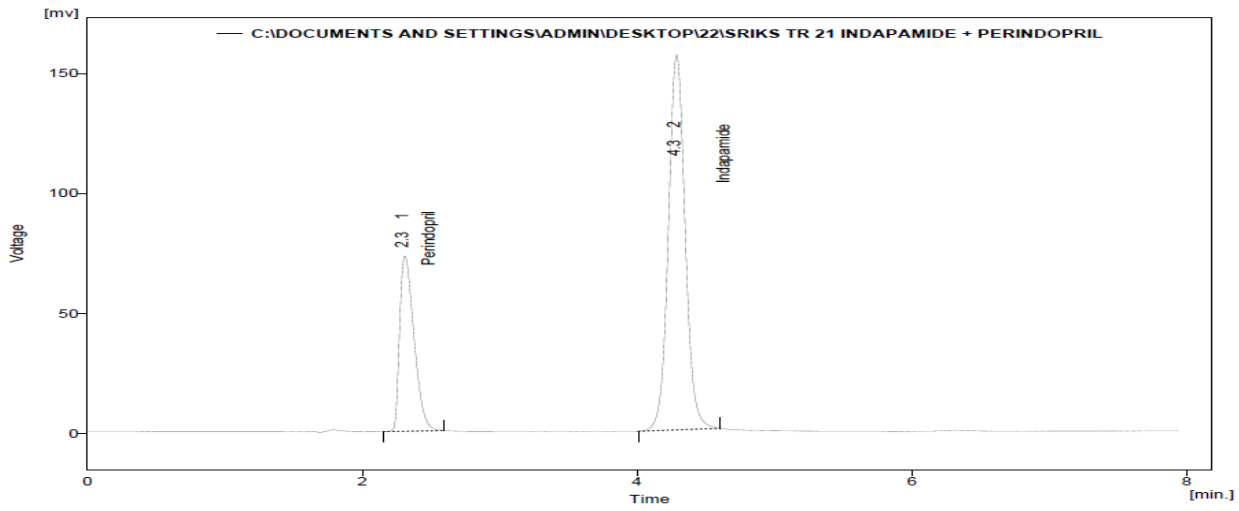
7.RESULTS AND DISCUSSIONS

7.1.ANALYTICAL METHOD DEVELOPMENT FOR THE ESTIMATION OF INDAPAMIDE AND PERINDOPRIL ERBUMINE BY RP-HPLC METHOD

Selection of mobile phase

Several solvent systems were tried to get good optimum resolutions of Indapamide and Perindopril erbumine in the chromatogram. The chromatogram is presented below.

Figure 1. Chromatogram showing peak separation with Buffer (Ammonium dihydrogen phosphate) pH 2.5 with ortho phosphoric acid : Acetonitrile (60:40), as mobile phase



Report

It was found that peaks of Indapamide and Perindopril erbumine were well resolved with the solvent system of Acetonitrile and Buffer (pH 2.5) in the ratio 40:60 with ortho phosphoric acid.

7.2. METHOD VALIDATION PARAMETERS

7.2.1. SYSTEM SUITABILITY

This parameter ensures that the analytical system is working properly and can give accurate and precise results. A 20µl solution of Indapamide and Perindopril erbumine solution was injected and the parameters like theoretical plates per column and tailing factor were calculated.

Figure 2. System suitability chromatogram for Indapamide and Perindopril erbumine

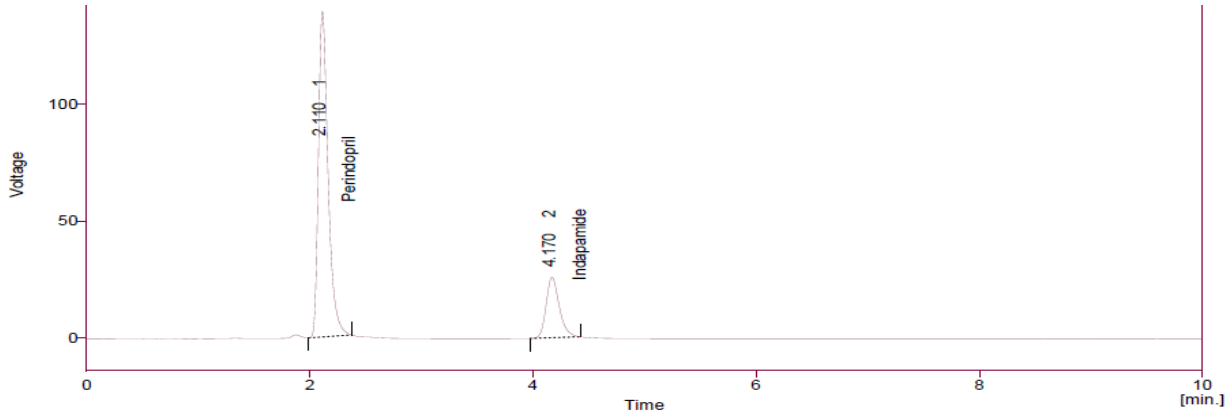


Table 1.System suitability parameter for Indapamide and Perindopril erbumine

Parameters	Indapamide	Perindopril erbumine	Acceptance criteria
Theoretical plates	6004	2831	NLT 2000
Tailing factor	1.3887	1.750	NMT 2.0
Height	25.88	138.81	-
Resolution	11.020		

Report

The system suitability parameters were developed for both Indapamide and Perindopril erbumine were found to be within acceptance criteria.

7.2.2.LINEARITY

The linearity parameter was performed to ensure that the test results are directly proportional to the concentration of analyte sample. For the linearity 20µl of each of working standard solution of Indapamide and Perindopril erbumine were injected into HPLC system. The peak area and concentration were plotted to get a standard calibration curve. The correlation coefficient was calculated. The results obtained were tabulated below.

Table 2. Linearity of Indapamide and Perindopril erbumine

S	Indapamide	Perindopril erbumine

S. No	Concentration (mcg/ml)	Peak area	Concentration (mcg/ml)	Peak area
1	2	41.899	10	184.717
2	4	81.739	20	334.557
3	6	121.039	30	502.17
4	8	162.628	40	670.6
5	10	204.908	50	836.134
6	12	239.872	60	972.623

Table 3. Linearity Report of Indapamide and Perindopril erbumine

Parameter	Acceptance criteria	Result observed	
		Indapamide	Perindopril erbumine
Correlation co-efficient	NLT 0.996	0.999	0.999

Figure 3. Standard calibration curve for Indapamide

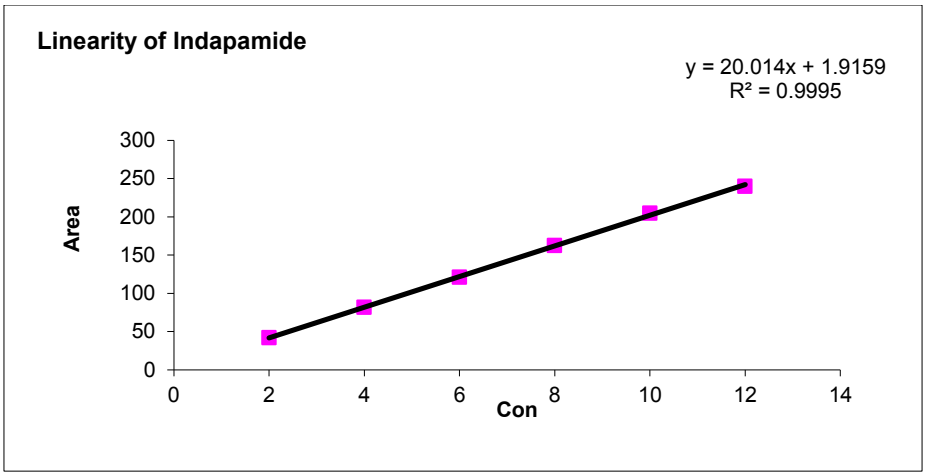


Figure 4. Standard calibration curve of Perindopril erbumine

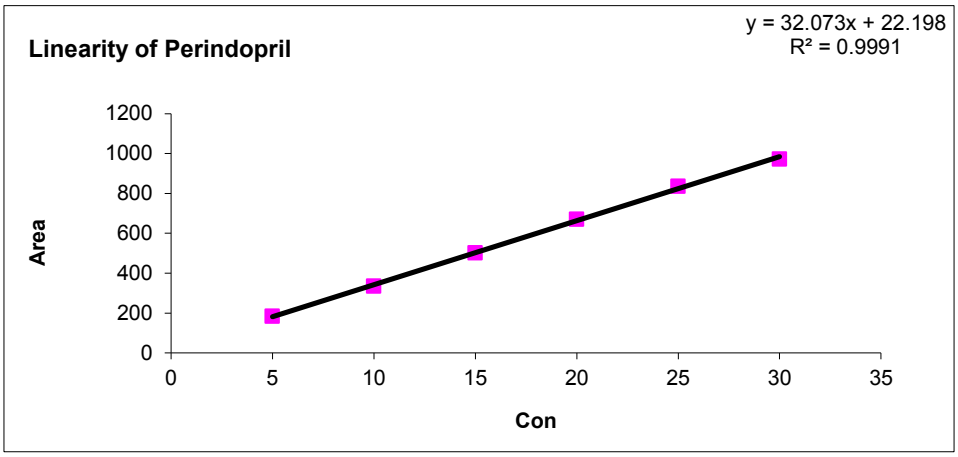


Table 4. Linearity of Indapamide and Perindopril erbumine

Parameter	**Results**	
	Indapamide	**Perindopril erbumine**
Slope	20.01	32.07
Intercept	+1.915	+22.19
Correlation co-efficient	0.999	0.999
Percentage curve fitting	99.9%	99.9%

Figure 5. Chromatogram for Linearity of Indapamide and Perindopril erbumine level 1

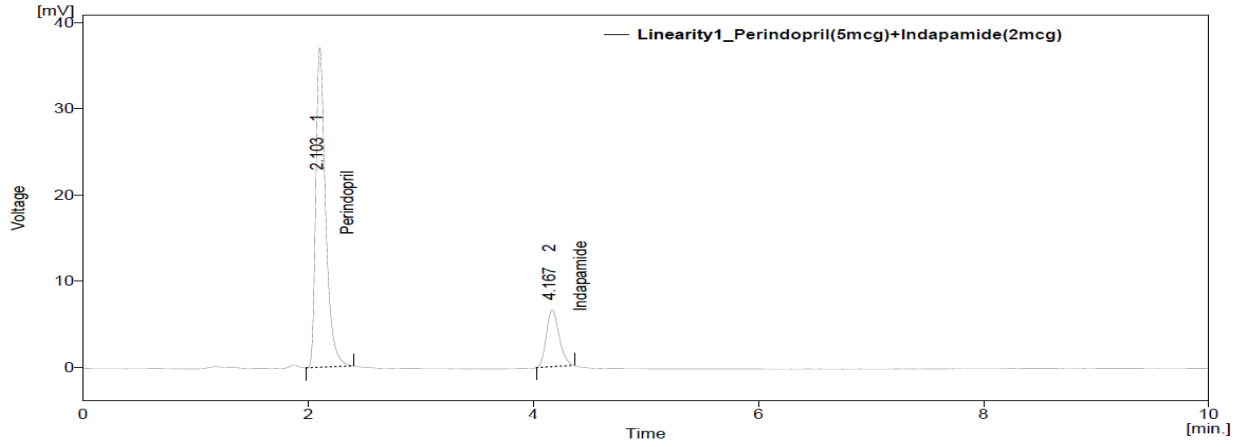


Figure 6. Chromatogram for Linearity of Indapamide and Perindopril erbumine level 2

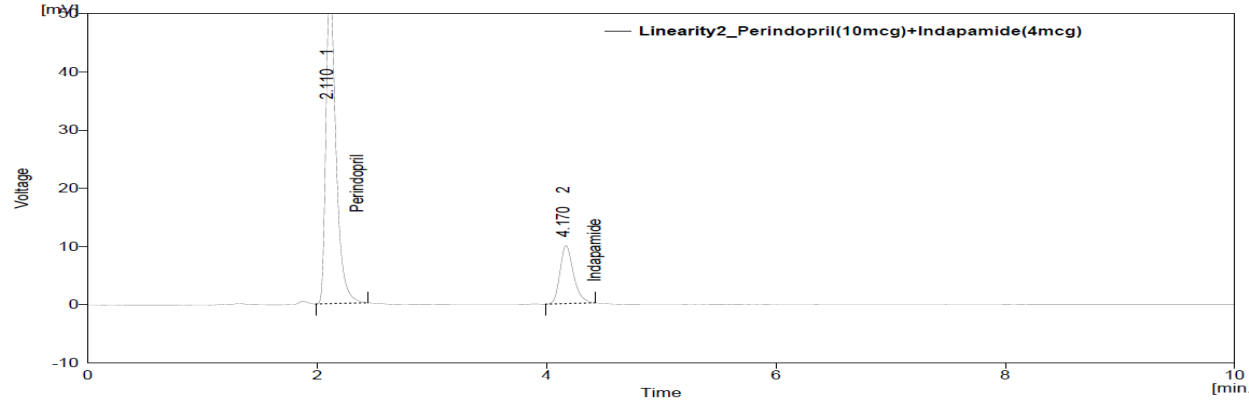


Figure 7. Chromatogram for Linearity of Indapamide and Perindopril erbumine level 3

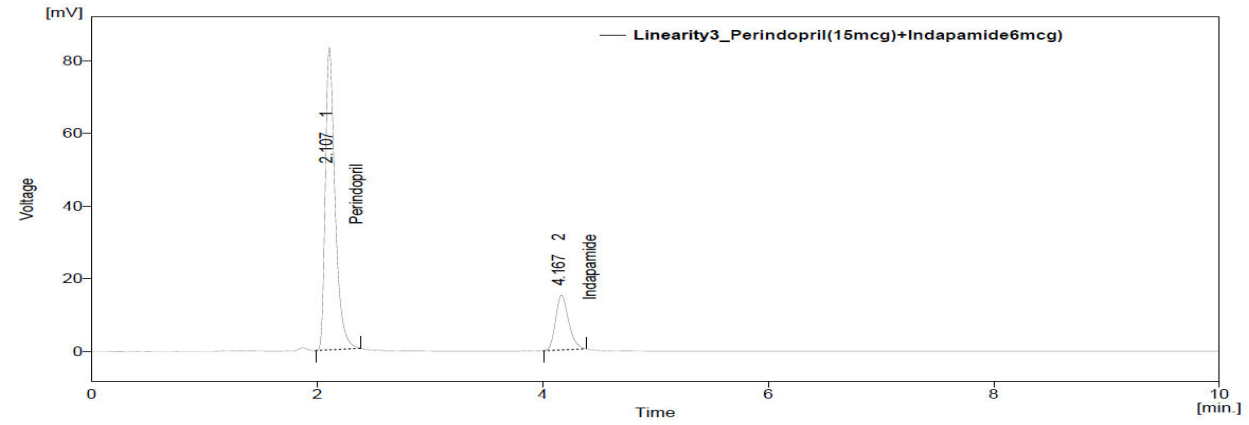


Figure 8. Chromatogram for Linearity of Indapamide and Perindopril erbumine level 4

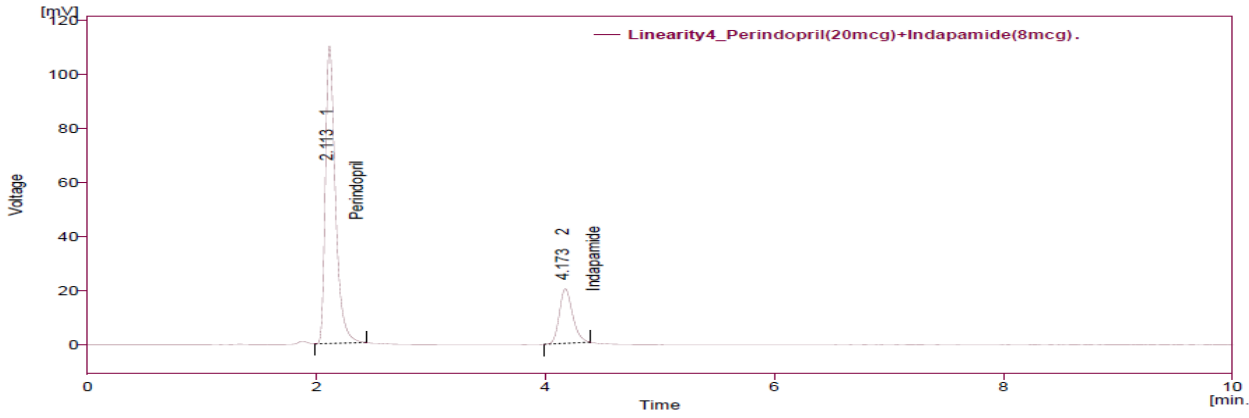


Figure 9. Chromatogram for Linearity of Indapamide and Perindopril erbumine level 5

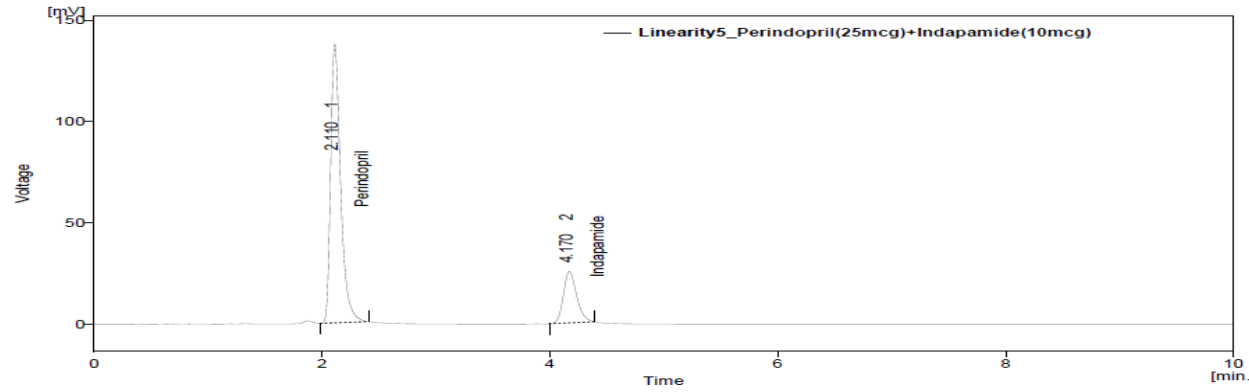


Figure 10. Chromatogram for Linearity of Indapamide and Perindopril erbumine level 6

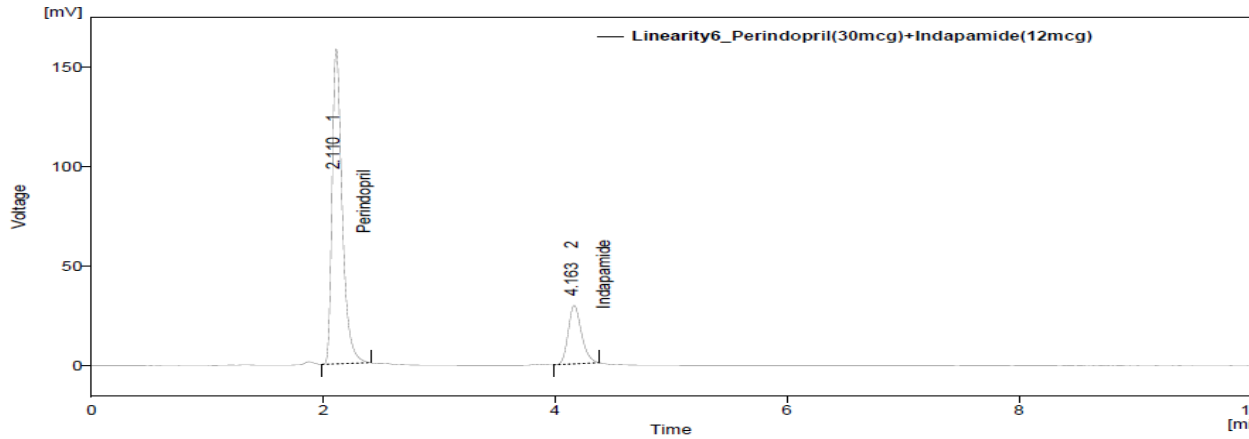
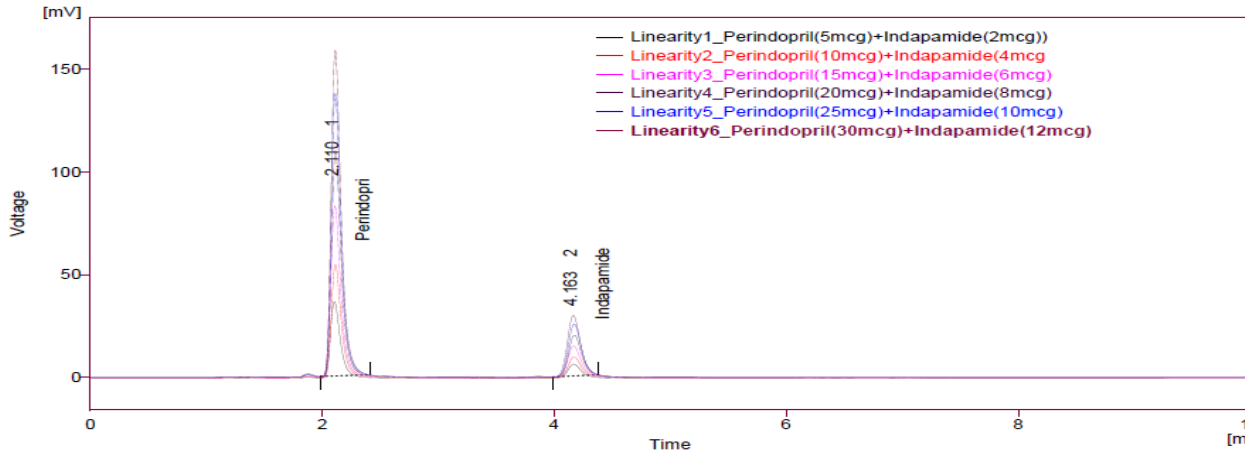


Figure 11. Linearity Report for Indapamide and Perindopril erbumine



Report

The proposed method is found to be linear at concentration of 2-12µg/ml for Indapamide and 5-30 µg/ml for Perindopril erbumine. The correlation coefficient and percentage curve fitting for Indapamide and Perindopril erbumine was found to be 0.999, 0.999 and 99.9%, 99.9% respectively which are well in the acceptance criteria limits.

7.2.3. Accuracy

This parameter is performed to determine the closeness of test results with that of true values which is expressed as % recovery.

Table 5. Accuracy of Indapamide

	Concentration µg/ml	Sample Area	Amount added	Recovery (µg)		% Recovery
				Individual value	Average value	
	8.55	21		0.786	0.7764	

		2.99	0.95			99.90
	8.55	210.804	0.95	0.7832		
	8.55	212.475	0.95	0.7600		
	10.45	193.134	0.95	0.8087	0.8002	99.59
	10.45	192.289	0.95	0.775		
	10.45	186.637	0.95	0.817		
	12.35	234.723	0.95	0.929	0.9350	98.48
	12.35	225.118	0.95	0.937		
	12.35	237.181	0.95	0.9392		

Table 6. Accuracy of Perindopril erbumine

	Concentration µg/ml	**Sample Area**	**Amount added**	**Recovery (µg)**		**% Recovery**
				Individual value	**Average value**	

	22.5	841.021	2.5	2.043	2.042	99.89
	22.5	839.021	2.5	2.036		
	22.5	842.437	2.5	2.048		
	27.5	756.279	2.5	2.486	2.493	99.77
	27.5	753.661	2.5	2.4966		
	27.5	758.107	2.5	2.499		
	32.5	920.161	2.5	2.949	2.952	99.96
	32.5	923.778	2.5	2.957		
	32.5	924.714	2.5	2.952		

Figure 12. Accuracy of standard preparation of Indapamide and Perindopril erbumine

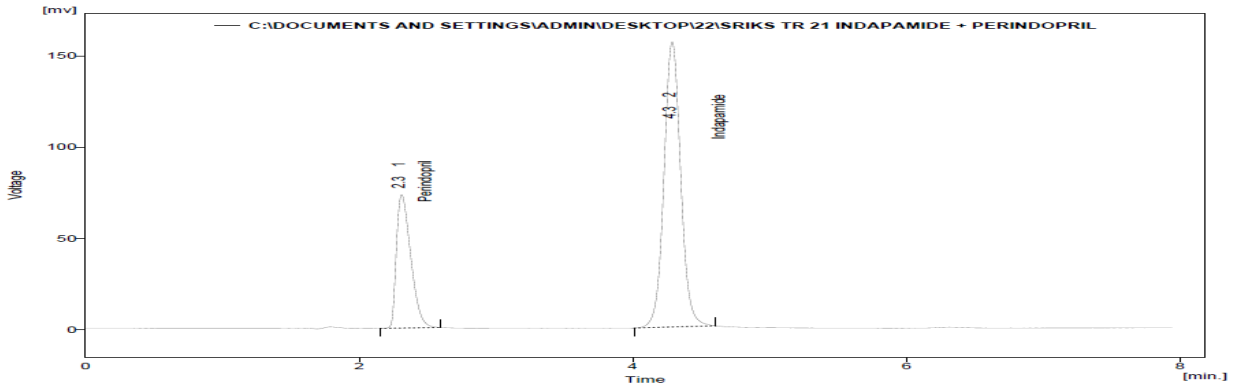


Figure 13. Accuracy of sample preparation of Indapamide and Perindopril erbumine

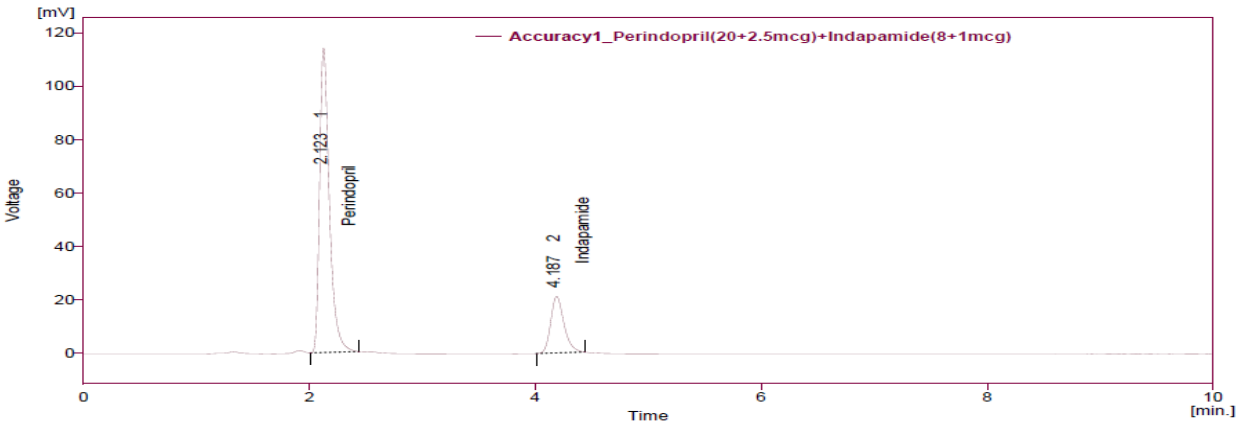


Figure 14. Accuracy of sample preparation of Indapamide and Perindopril erbumine

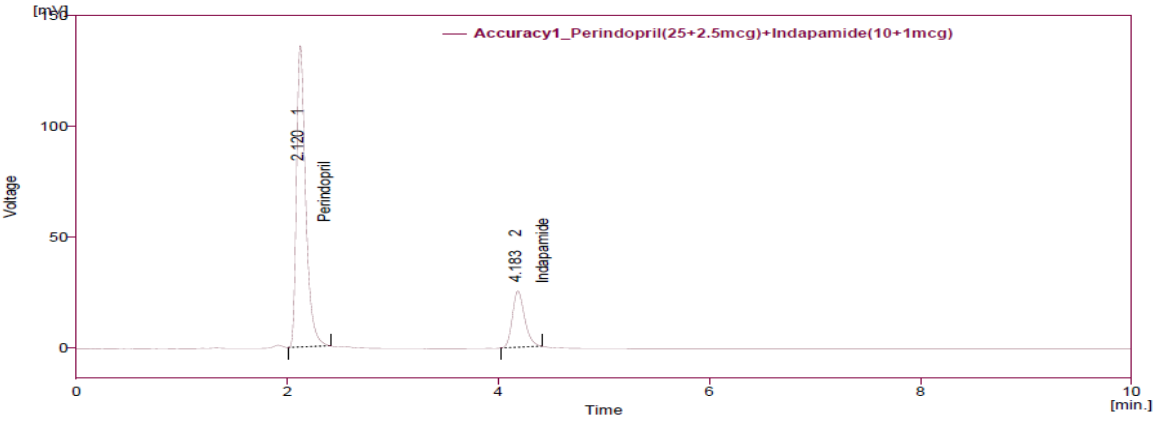
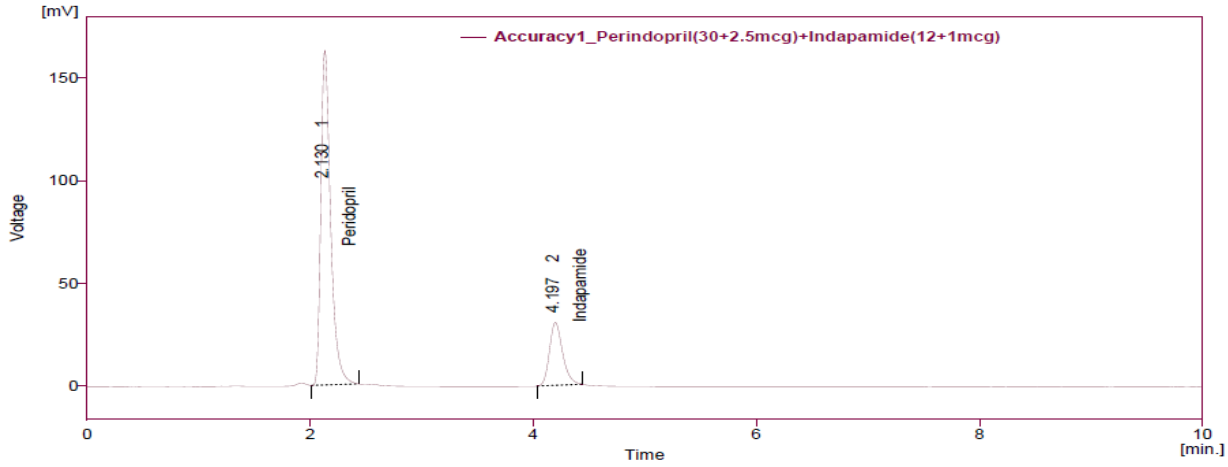


Figure 15. Accuracy of sample preparation of Indapamide and Perindopril erbumine



Report

The mean percentage recovery for Indapamide and Perindopril erbumine was found to be between 98.48- 99.90 and 99.89-99.96 respectively, which are well within the acceptance criteria and hence the method was found to be accurate.

7. 2.4. System Precision

This method validation parameter was performed to ensure the closeness of results between true value and experimental value. Five injections of 20µl volume Indapamide and Perindopril erbumine respectively were injected into the system. The retention time and peak area were recorded and are presented below.

Table 7. Precision of Indapamide and Perindopril erbumine

Sl. No	Injection number	Indapamide		Perindopril erbumine	
		Retention	Area(mVs)	Retention	Area(mVs)

	(100 µg/ml)	time(min)		time(min)	
1	Injection-1	4.170	211.427	2.110	838.37
2	Injection-2	4.173	210.662	2.113	837.524
3	Injection-3	4.173	209.677	2.117	837.314
4	Injection-4	4.173	208.992	2.117	837.410
5	Injection-5	4.18	211.089	2.117	837.823
	AVRG	4.1738	210.3694	2.1148	837.6882
	STDEV	0.003701	1.012251	0.003194	0.42644
	%RSD	0.09	0.48	0	0.05

Figure 16. Precision of Indapamide and Perindopril erbumine (injection 1)

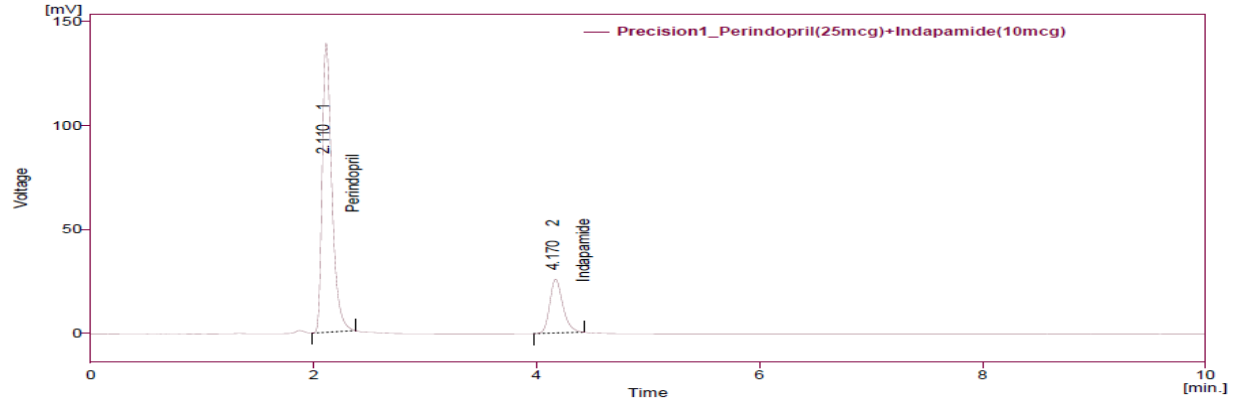


Figure 17. Precision of Indapamide and Perindopril erbumine (injection 2)

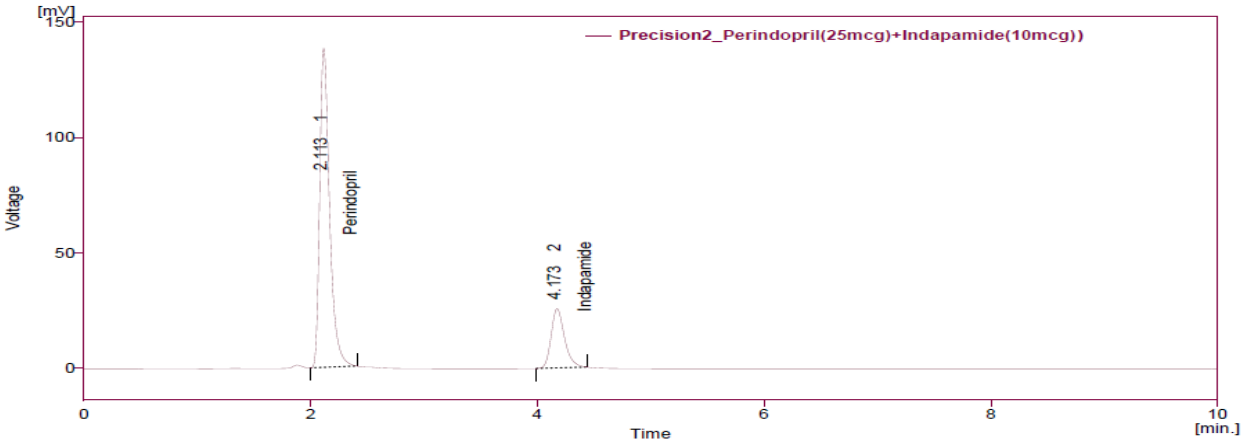


Figure 18. Precision of Indapamide and Perindopril erbumine (injection 3)

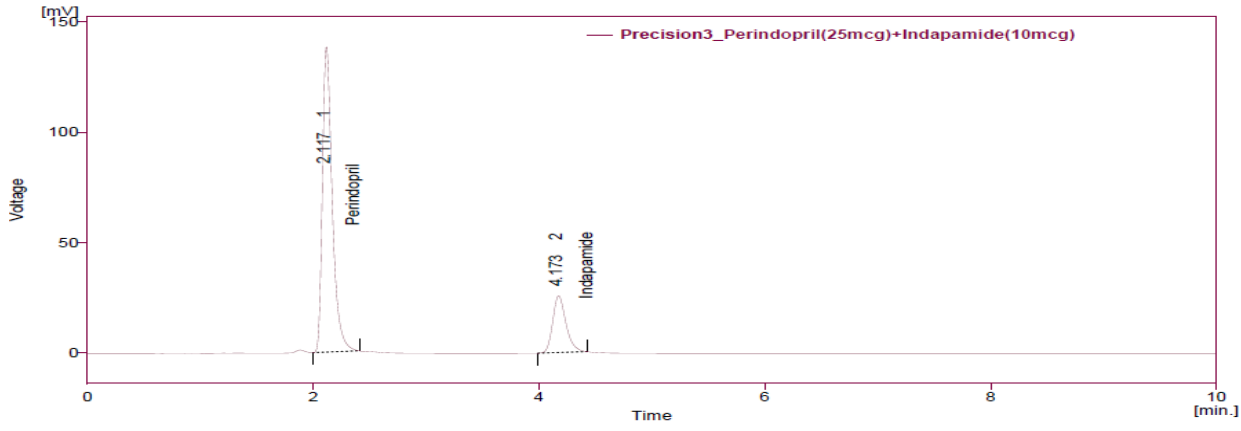


Figure 19. Precision of Indapamide and Perindopril erbumine (injection 4)

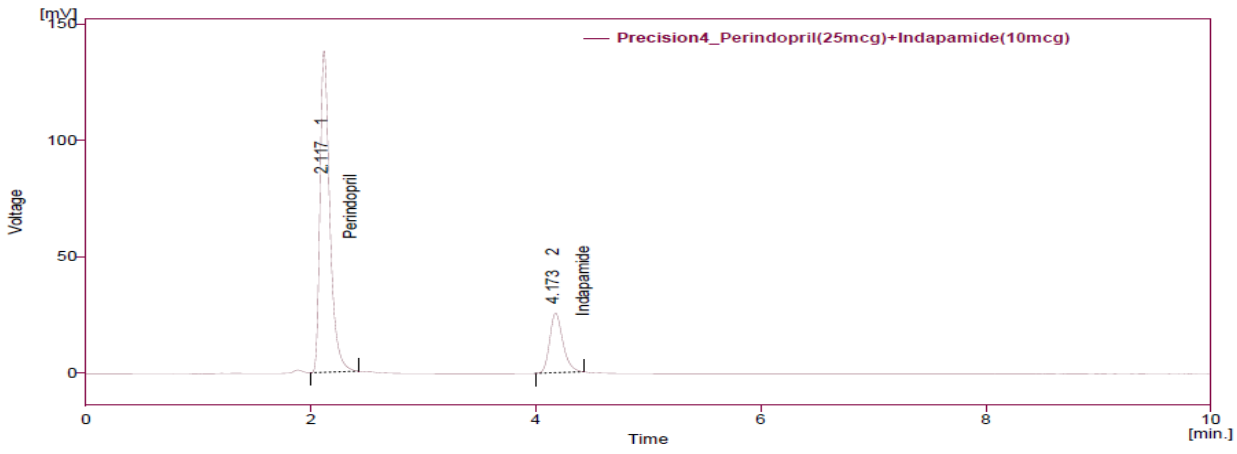


Figure 20. Precision of Indapamide and Perindopril erbumine (injection 5)

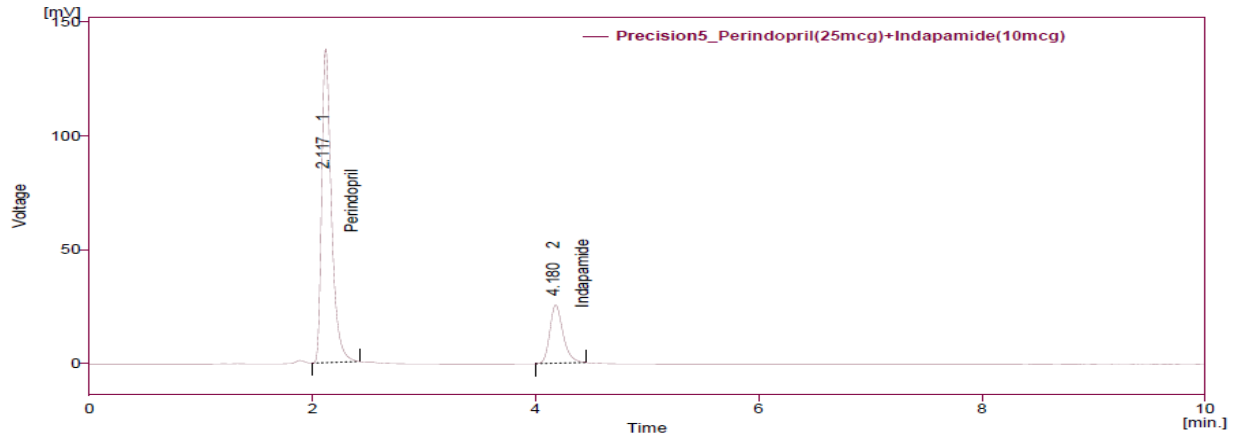


Table 8. Precision of Indapamide and Perindopril erbumine

Drug	%RSD for RT	%RSD for Peak area	Accepatance criteria
Indapamide	0.09	0	NMT 2%
Perindopril erbumine	0.48	0.05	NMT 2%

Report

The %RSD values of retention time and Peak area for five injections of Indapamide and Perindopril erbumine were found to be 0.09, 0.48 and 0.0, 0.05 respectively which are well within acceptance criteria limit.

7.2.5. Method Precision

The method precision was performed to standardize methodology i.e., to check whether the developed method is precise. Six injections of 20µl working standard solutios of concentration (200:200)µg/ml of Indapamide and Perindopril erbumine were injected. Chromatograms were recorded and presented below. The %RSD for peak area was calculated.

Sl.No	**Injection number (100 mcg/ml)**	**Indapamide**		**Perindopril erbumine**	
		Retention time	**Peak Area**	**Retention time**	**Peak Area**
1	Injection-1	4.190	209.916	2.123	835.047
2	Injection-2	4.187	210.938	2.123	835.956
3	Injection-3	4.180	210.845	2.120	837.987
4	Injection-4	4.187	207.862	2.123	834.225
5	Injection-5	4.190	210.260	2.123	839.131
	AVRG	4.1868	209.9642	2.1224	836.4692
	STDEV	0.004087	1.248315	0.001342	2.043976
	%RSD	0.10	0.59	0.06	0.24

Table 9. Method Precision of Indapamide and Perindopril

Figure 21. Method Precision of Indapamide and Perindopril erbumine(injection 1)

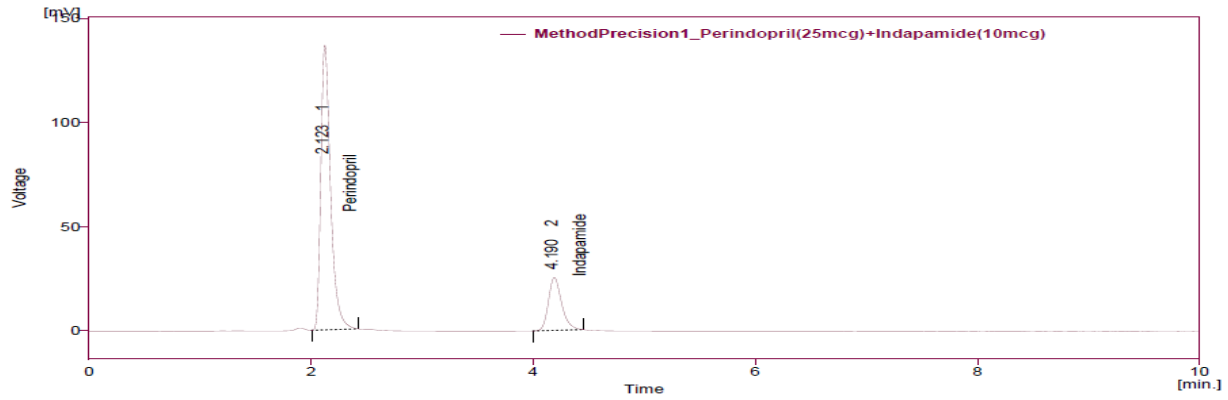


Figure 22. Method Precision of Indapamide and Perindopril erbumine (injection 2)

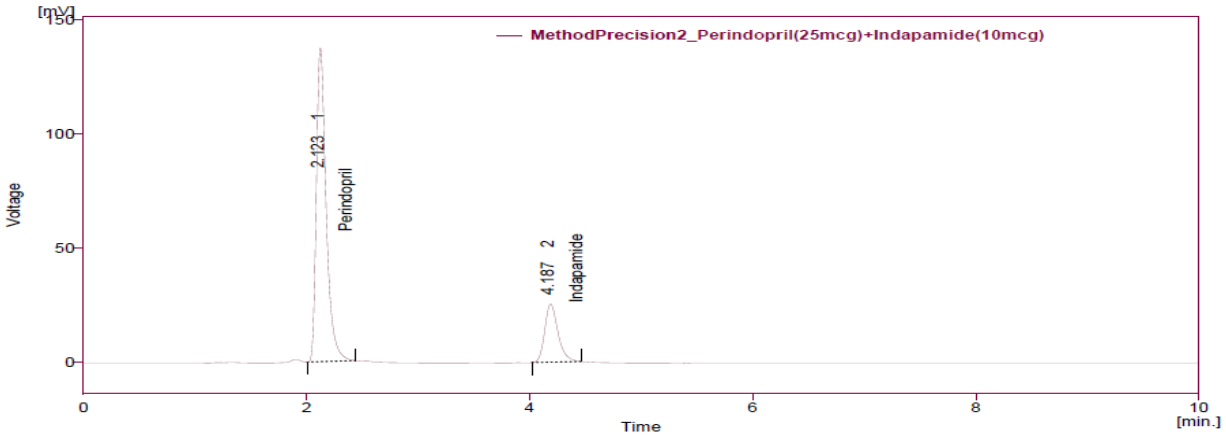


Figure 23. Method Precision of Indapamide and Perindopril erbumine (injection 3)

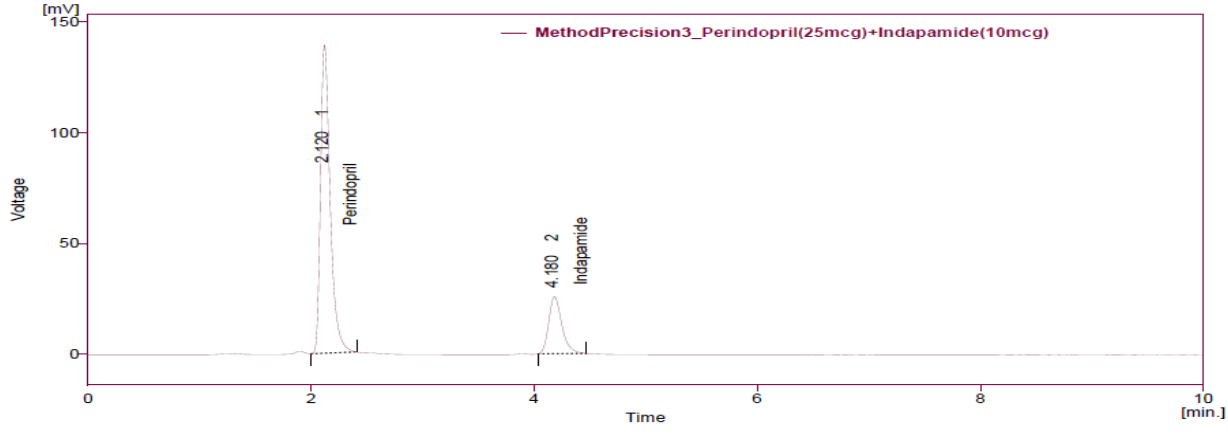


Figure 24. Method Precision of Indapamide and Perindopril erbumine (injection 4)

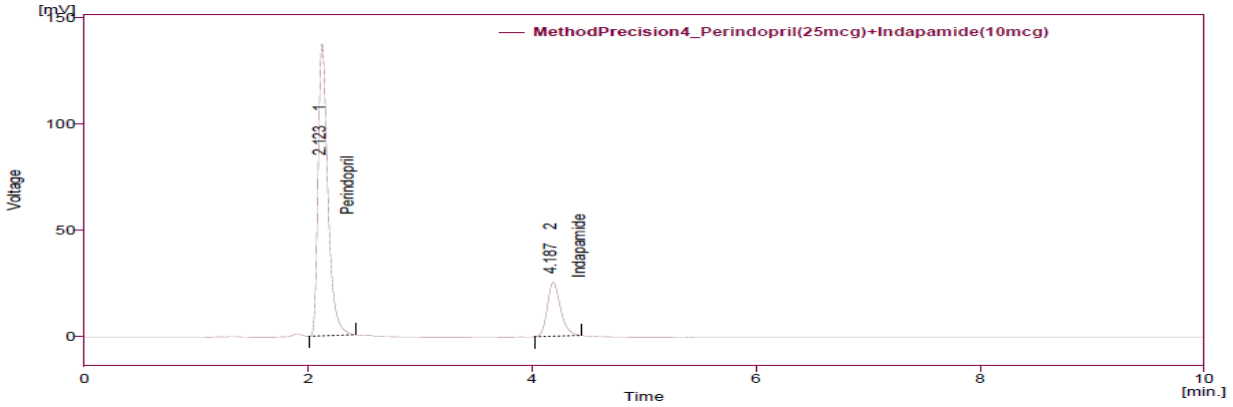


Figure 25. Method Precision of Indapamide and Perindopril erbumine (injection 5)

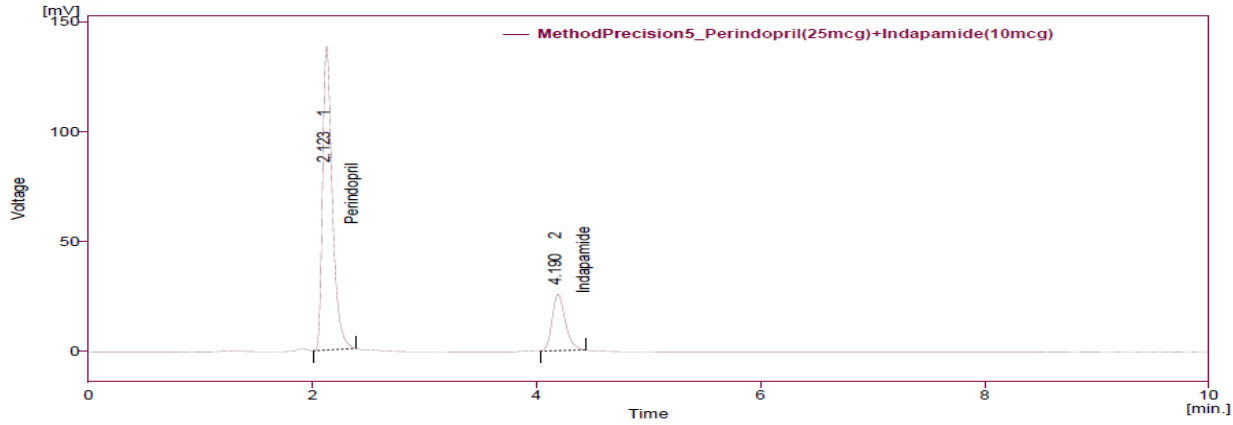


Table 10. Method precision of Indapamide and Perindopril erbumine

Drug	%RSD of Peak Area	Acceptance criteria
Indapamide	0.59	NMT 2%
Perindopril erbumine	0.24	NMT 2%

Report

The %RSD values for Retention time and Peak area for five injections of Indapamide and Perindopril erbumine were found to be 0.10, 0.06 and 0.59, 0.24 respectively, which are well within acceptance criteria.

7.2.6.Robustness

This parameter was carried out to check the ability of the system to give unaffected results for small deliberate changes in system parameters and method parameters.

Table 11. Robustness of Indapamide and Perindopril erbumine

Proposed variation		Indapamide	Perindopril erbumine	Acceptance criteria
		Asymmetry factor		
Variation in flow rate	0.9ml	0.137	0.100	In between 0.5 and 2.0
	1.1ml	1.345	1.833	
Variation in wavelength	218nm	1.367	1.750	
	224nm	1.387	1.750	

Figure 26. Robustness of Indapamide and Perindopril erbumine (0.9ml)

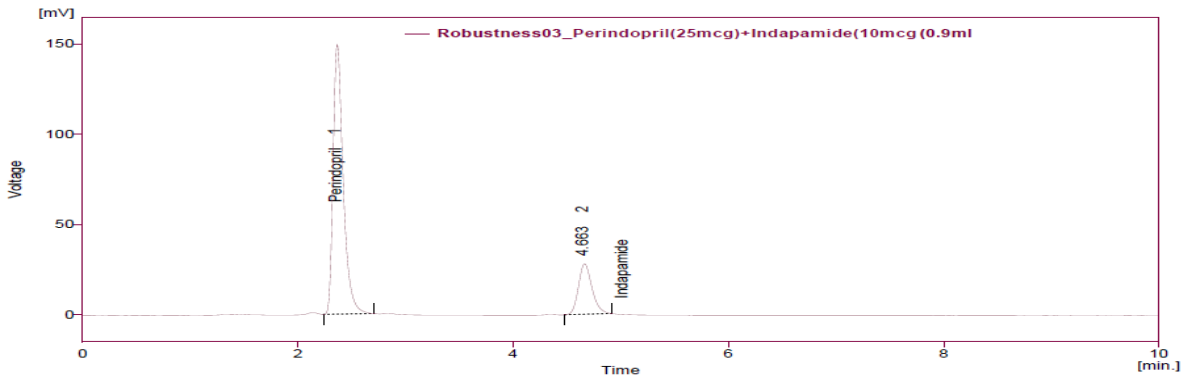


Figure 27.Robustness of Indapamide and Perindopril erbumine (1.1ml)

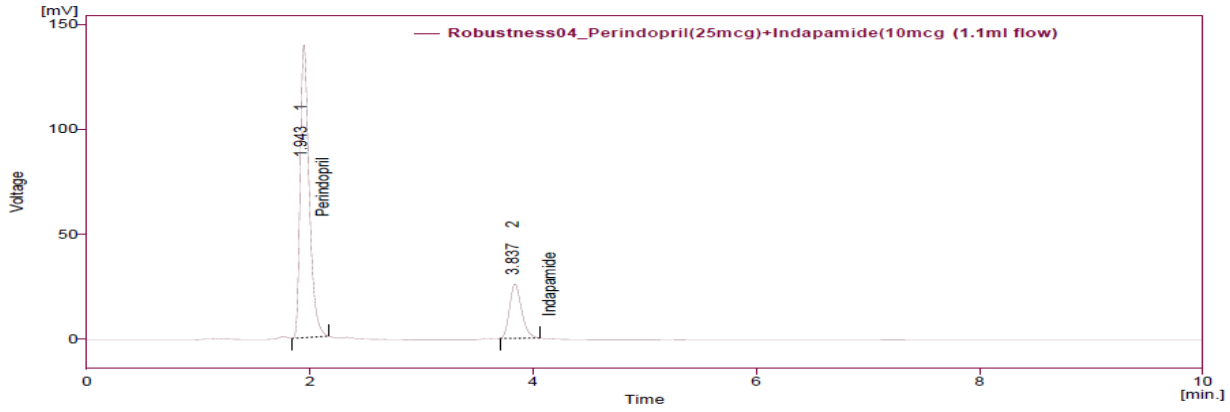


Figure 28. Robustness of Indapamide and Perindopril erbumine (218 nm)

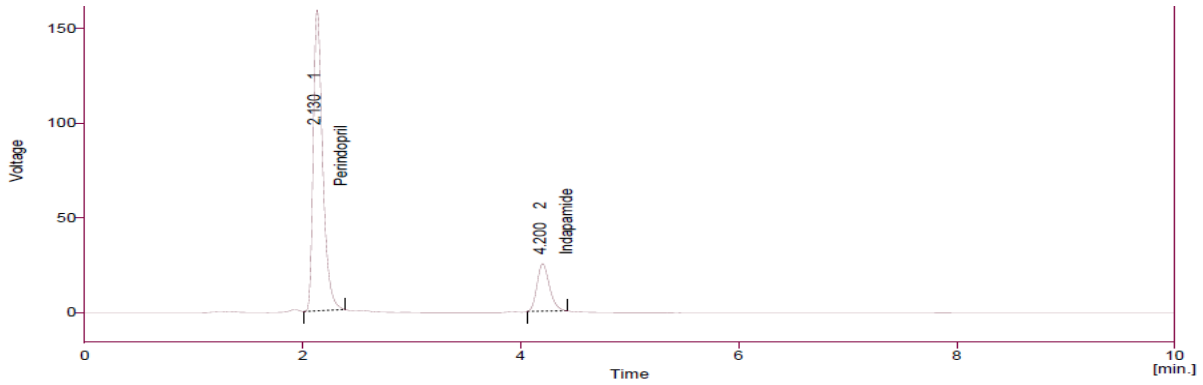
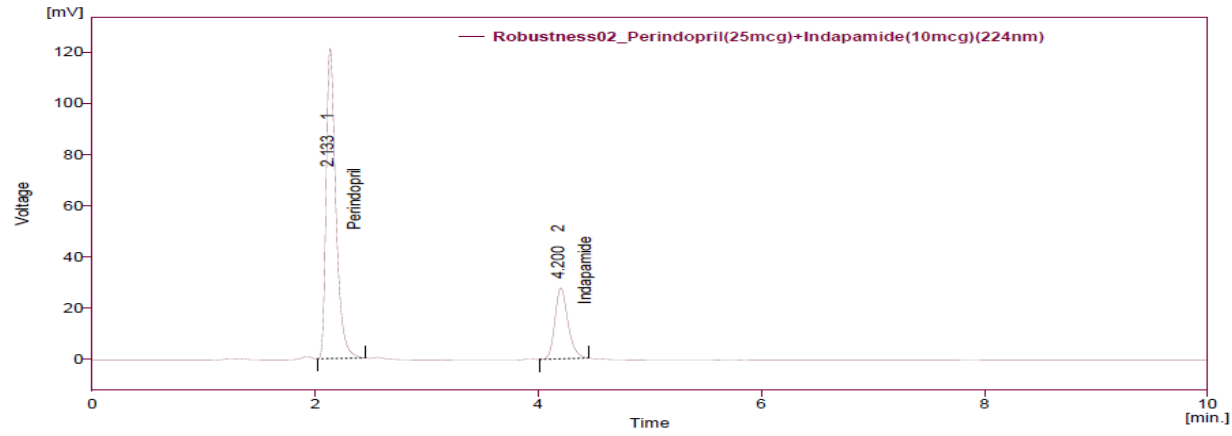


Figure 29. Robustness of Indapamide and Perindopril erbumine (224 nm)



Report

From the above observations it can be concluded that, the method is robust with respect to change in flow rate and wavelength.

7.2.7. Ruggedness (Reproducibility)

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments.

Table 12. Ruggedness of Indapamide and Perindopril erbumine

	Indapamide		Perindopril erbumine	
	Retention time	**Peak Area**	**Retention time**	**Peak Area**
Analyst(1)(100mcg)	4.180	211.089	2.117	837.823
Analyst(2)(100mcg)	4.180	211.089	2.117	837.823
AVRG	4.810	211.089	2.117	837.823

Figure 30.Ruggedness of Indapamide and Perindopril erbumine (Analyst 1)

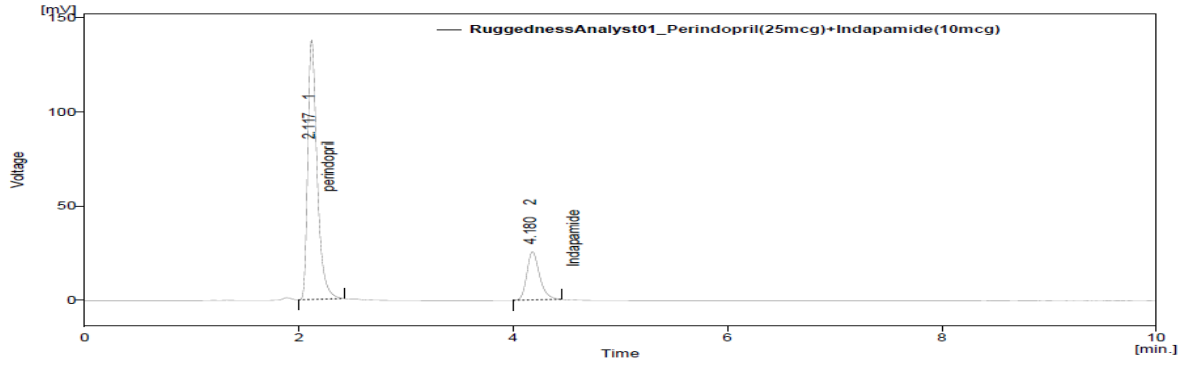
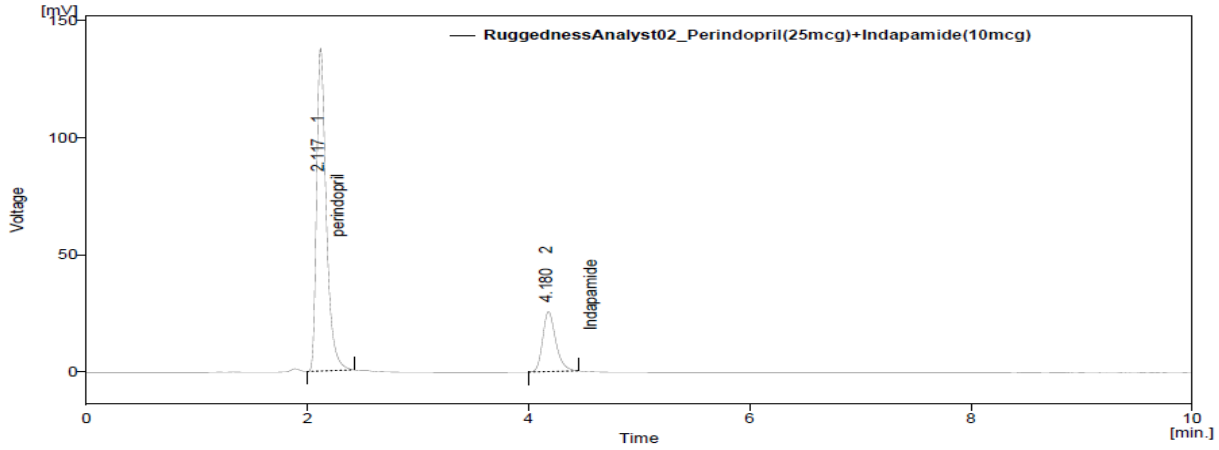


Figure 31. Ruggedness of Indapamide and Perindopril erbumine (Analyst 2)



Report

The results are within the acceptance limit, the proposed method is found to be rugged.

7.2.8.Specificity

This parameter was performed to assess and ensure that the impurities, degraded products and diluents do not affect the samples analyzed. 20µl of diluents, Indapamide, Perindopril erbumine were injected into the system and chromatograms recorded and presented below.

Figure 32. Specificity of Indapamide and Perindopril erbumine in 0.1 M HCl

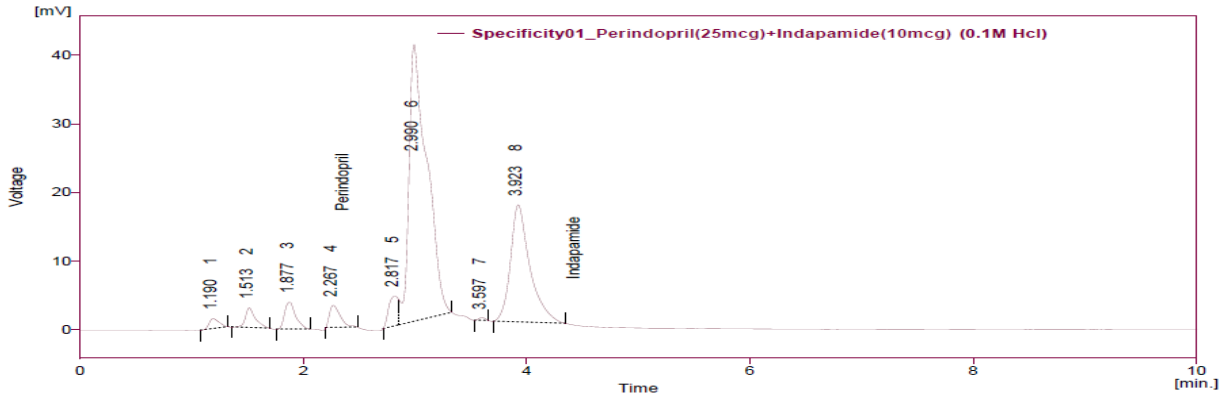
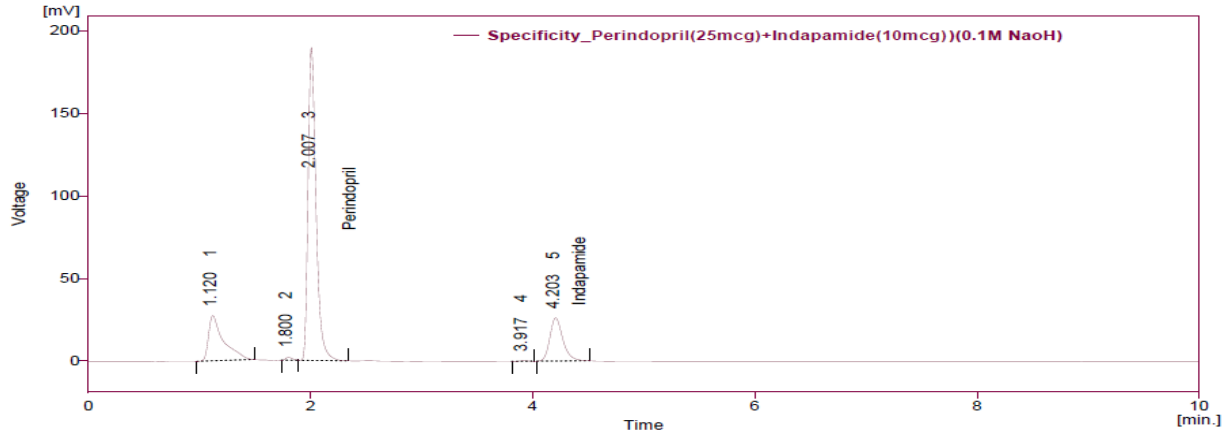


Figure 33. Specificity if Indapamide and Perindopril erbumine in 0.1N NaOH



Report

The chromatogram of Indapamide and Perindopril erbumine were analyzed and there is no interference from diluents, excipients and impurities with peaks of Indapamide and Perindopril erbumine.

7.2.9.ASSAY

The developed and valideated HPLC method was used to determine Indapamide and Perindopril erbumine in combined dosage form (tablets).

Formulation used : Tablet

Brand Name : Periguard-G

Company Name : Primal Health care Ltd., Mankind pharma

Procedure : 20µl of the sample solutions were injected into HPLC system and the

results obtained are presented below.

Table 13. Assay of Indapamide and Perindopril erbumine by RP-HPLC

S.no	Parameter	Indapamide	Perindopril erbumine
01	Spl. Area	312.0265	870.8355
02	Std.Area	322.4047	929.659
03	Std. Wt	5.1mg	13.4mg
04	Spl.Wt	1010.6mg	1010.6mg
05	LC	0.625mg	1.569mg
06	Avg.Wt	126.3mg	126.3mg
07	Std.Purity	99.84%	99.84%
08	Assay %	99.17078	99.82123

Indapamide				Perindopril erbum		
Standard Rt	**Sample Rt**	**Standard area**	**Sample area**	**Standard Rt**	**Sample Rt**	**Sta are**
2.1271	2.197	222.595	315.616	4.190	4.143	865
2.1271	2.184	225.793	312.437	4.193	4.157	868
2.1271	2.1905	224.194	314.0265	4.1915	4.150	866
0	0.009192	2.261327	2.247892	0.002121	0.002828	2.6
0.0	0.42	1.01	0.72	0.05	0.07	0.3

Table 14. Assay of Indapamide and Perindopril erbumine by RP-HPLC

Figure 34. Chromatogram for assay of Indapamide and Perindopril erbumine

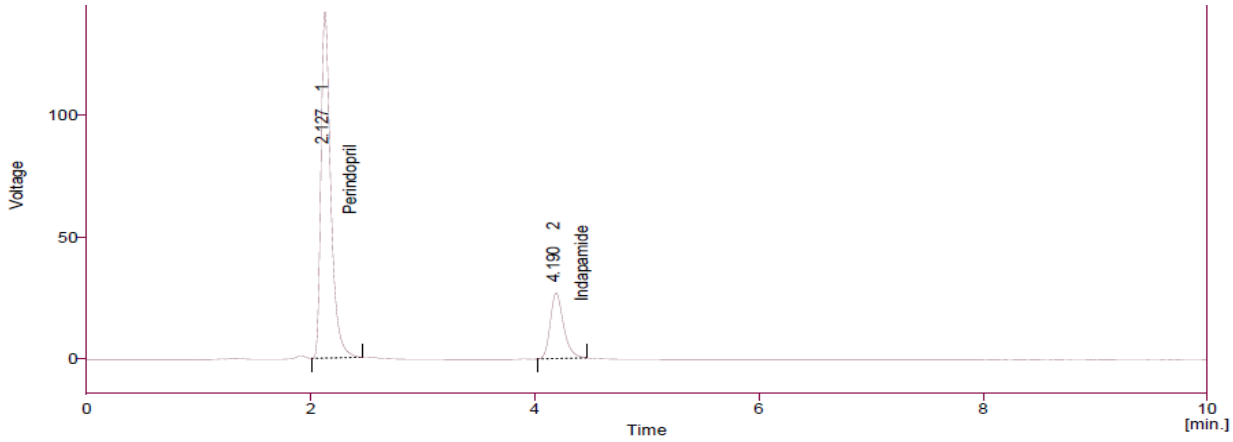
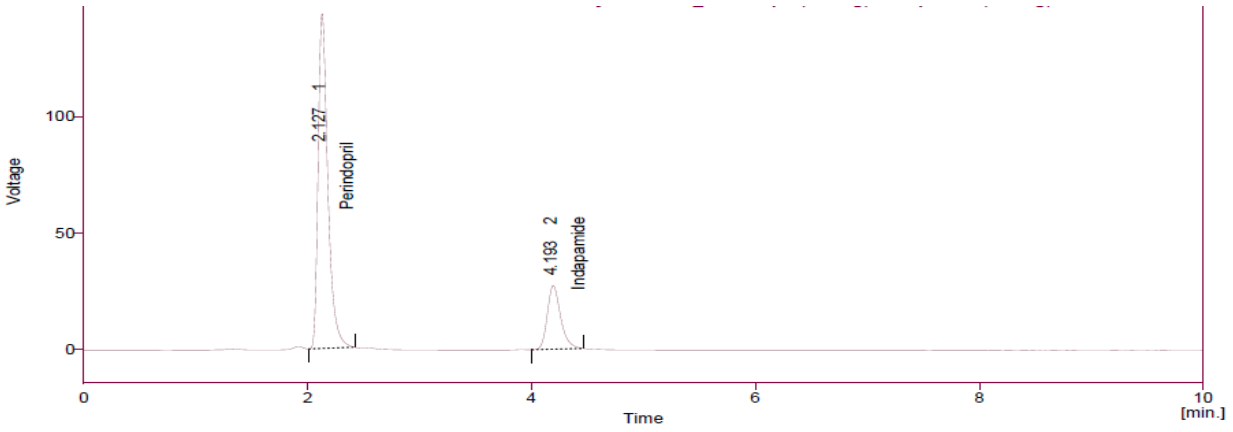


Figure 35. Chromatogram for assay of Indapamide and Perindopril erbumine



Report

The percentage purity of Indapamide and Perindopril erbumine in Periguard-G tablets was found to be in the range.

8. SUMMARY

8.1. Table for Validation parameters of RP-HPLC

SNo	Parameters	Limit	Proposed HPLC method	
			Indapamide	Perindopril erbumine
1.	Wave length (nm)	-	240 nm	210 nm
2.	Linearity	-	2-12µg/ml	5-30 µg/ml
3.	Regression equation	-	y=20.01x+ 1.915	Y=32.07x+22. 19
4.	Correlation coefficient	NLT 0.996	0.999	0.999
5.	Slope	-	20.01	32.07
6.	Intercept	-	+1.915	+22.19
7.	System Precision	RSD NMT 2%	0.48	0.05
8.	Method Precision	RSD NMT 2%	0.59	0.24
9.	% Recovery	98-102%	99.54	99.76

System suitability parameters were determined. The number of theoretical plates per column for Indapamide and Perindopril erbumine was found to be 6004 and 2831 respectively. The symmetry factor or tailing factor was found to be 1.3887 and 1.750 for Indapamide and Perindopril erbumine. The resolution of the method was calculated and was found to be 11.020.

Specificity of the method was determined. The chromatogram of Indapamide and Perindopril erbumine were analyzed and there is no interference from diluents, excipients and impurities with peaks of Indapamide and Perindopril erbumine.

Linearity of the drugs response was found to be in the concentration range of 2-12µg/ml for Indapamide and 5-30 µg/ml for Perindopril erbumine. The correlation coefficient and percentage curve fitting for Indapamide and Perindopril erbumine was found to be 0.999, 0.999 and 99.9%, 99.9% respectively which are well in the acceptance criteria limits.

Precision of the system and method was determined. The %RSD values of retention time and Peak area for five injections of Indapamide and Perindopril erbumine were found to be 0.09, 0.48 and 0.0, 0.05 respectively which were well within acceptance criteria limit for system precision. The %RSD values for Retention time and Peak area for five injections of Indapamide and Perindopril erbumine were found to be 0.10, 0.06 and 0.59, 0.24 respectively, which were well within acceptance criteria for method precision. Hence the proposed method was found to provide high degree of precision and reproducibility.

Accuracy was determined through recovery studies of Indapamide and Perindopril erbumine. The mean percentage recovery for Indapamide and Perindopril erbumine was found to be between 98.48- 99.90 and 99.89-99.96 respectively, which were well within the acceptance criteria and hence the method was found to be accurate, indicating no interference of the drugs with each other or with the excipients present in the formulation.

Robustness of the method was determined by changing the flow rate of mobile phase slightly in RP-HPLC method. The %RSD of peak area, tailing

factor and Theoretical plates were found to be well within the acceptance criteria.

Ruggedness or degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in analysts. The results are within the acceptance limit, the proposed method is found to be rugged.

Percentage purity of Indapamide and Perindopril erbumine in combination was determined and percentage purity was found to be in the 99.17078 and 99.82123 for Indaapmide and Perindopril erbumine.

9.Conclusion

A RP-HPLC method was developed and validated successfully for the estimation of Indapamide and Perindopril erbumine in bulk and tablet dosage formulation. The methods were found to be accurate, precise, linear, specific and reproducible for the simultaneous determination of Indapamide and Perindopril erbumine in bulk and tablet dosage form (tablets).

Hence these methods can be used for simultaneous estimation of Indapamide and Perindopril erbumine in routine table.

Future scope

This study can be extended by studying the degradation kinetics of Indapamide and Perindopril erbumine determination by second order derivative and simultaneous UV method, HPTLC method and also its estimation in plasma and biological fluids.

BIBLIOGRAPHY

1.British Pharmacopoeia, London: British Pharmacopoeial Commission Office; 2010. p. 1642.

2.The United States of Pharmacopoeia-National Formulary-25. Asian Edition, United 2007; vol. II: 2340.

3. A.K. Pathak Rachana Lodhi,1 Mukesh K. Shikhri., 2011, A Selective Reverse Phase HPLC Method For Simultaneous Determination of Perindopril and Indapamide In Solid Dosage Form And Bulk Drug, Journal of Pharmacy Research, 4(8): pp 2512-2513.

4. Juddy joseph, Blessen, Dr. Sundarapandian., 2011, Simultaneous Estimation of Indapamide and Perindopril by RP_HPLC in pharmaceutical dosage forms, International Journal of Pharmacy and Pharmaceutical Sciences,3(4) pp 289-293.

5. Mohit G Dewani, Kailash G Bothara2, Ashwini R Madgulkar1 and Mrinalini C Damle., 2011, Simultaneous estimation of Perindopril erbumine and Indamide in Bulk and Tablet dosage forms by HPTLC, International Journal of Comprehensive Pharmacy, 1(01) pp 1-4.

6. Darshana K. Modi, Chagan N. Patel., 2011, Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Perindopril and Indapamide in Combined Dosage Form by Absorbance Correction Method, International Journal of PharmTech Research, 2(1), pp 411-416

7. Jignesh P. Prajapati M.B.Patel, Rashmika J. Prajapati, Nimesh A.Prajapat., 2011, Simultaneous Estimation of Perindopril erbumine and Amlodipine besylate by Absorption Correction method, International Journal of Applied Biology and Pharmaceutical Chemistry, 2(3), pp 230-233.

8. A.I. Patel, C.K. Oza, J.P. Prajapati, A.J.Vyas and P. mehta., 2011, RP-HPLC method for the Determination of Losartan Potassium and Perindopril

erbumine in combined Tablet dosage form, International Journal of Pharma and Bio Sciences, 2(1), pp 709-715.

9. Patel Amit R, Chandrul Kaushal Kishor.,2011, Method Development, Validation and Stability study for simultaneous estimation of Telmisartan and Indapamide by RP-HPLC in Pure and marketed formulations, international Journal on Pharmaceutical and Biomedical Research, 2(1), pp 4-16.

10.Darshana K. Modi et Chhagan N. Patel., 2011, Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Perindopril and Indapamide in Combined Dosage Form by Simultaneous Equation Method,
Eurasian Journal of Analytical Chemistry, 6(1), pp 46-52.

11. Prameela Rani, Bala Sekaran.C., 2009, A Validated Rp-HPLC method for the Determination of Perindopril erbumine in Pharmaceutical formulations, 1(3), pp 575-578.

12. Dewani Mohit G, Bothara kailash G, Damle Mrinalini., 2010, Development and Validation of stability indicating HPTLC method for the determination of Perindopril Erbumine, International Research Journal of Pharmacy, 1(1), pp 428-435.

13. Nevin Erk., 2001, Comparison of spectrophotometric and an LC method for the determination perindopril and indapamide in pharmaceutical formulations, Journal of Pharmaceutical and Biomedical Analysis, 26(2001) pp 43-52.